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PROGRESS REPORT  
of the  
EASTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION  
AGRICULTURAL RESEARCH SERVICE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between April 1, 1968, and March 31, 1969. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

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## INTRODUCTION

The mission of the Eastern Utilization Research and Development Division is to develop new and expanded markets for designated farm commodities, through research to develop new and improved products and processes based on these commodities. The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup and Eastern fruits and vegetables including potatoes.

In carrying out its mission, the Division does research in physical and biological science and in engineering throughout the spectrum of basic research, applied research and pilot plant development. Division scientists are aware of the key role that basic research plays in uncovering new information that may be later exploited in applied research and development. Hence, a substantial portion of the Division's effort is in basic research. When appropriate, engineers conduct pilot plant studies of promising laboratory developments to provide engineering and cost data essential to industrial application feasibility determinations.

The research effort at the Eastern Division amounts to approximately 195 scientist man-years. In addition, the Division supervises domestic research contracts and grants equivalent to 29.5 scientist man-years per year at 20 locations in the United States. The Division's program is supplemented by a variety of research projects in foreign countries under PL-480 grants. There are now a total of 29 such grants in 10 countries.

The Division is organized in 10 laboratories of which 5 are located entirely at the Eastern Regional Research Laboratory, Wyndmoor, Pa., one is at Beltsville, Md., with part of its research program at Wyndmoor, and one is at Washington, D. C., with part of its research program at Beltsville. One of the laboratories at Wyndmoor has some of its research located at East Grand Forks, Minnesota, and a second has research located at Lexington, Kentucky. One of the Division's laboratories is devoted to pioneering research on the biophysical mechanisms of macromolecules, and is located at Brandeis University, Waltham, Massachusetts.

In every phase of their research, Division scientists cooperate with representatives of colleges and universities, state experiment stations, research institutes and associations, industrial organizations and with other Government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. Currently 2 such agreements are in effect.

The farm products with which the Eastern Division deals provide more than half of the nation's cash farm receipts; the major part of U. S. farmland suitable for cultivation is used to provide feed for livestock and dairy cattle; in seven states tobacco provides more cash receipts than any other field crop. It is thus evident that maintaining and enlarging the markets

for these and the other farm products under study at the Eastern Division should be a major national concern.

Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Following are some examples of recent developments based on research at the Eastern Division and on Division-supervised research that typify such achievements.

Cockle, A Sheepskin Defect. Besides causing annual losses of at least \$4 million to the sheepskin segment of our leather industry, cockle seriously impairs both grain and suede types of leather, resulting in many products of inferior quality which invite replacement by substitutes. It is a widespread, seasonal skin condition that reaches its peak of severity in late winter and early spring, disappearing gradually with the advent of summer or after shearing. The defect occurs as hard, raised nodules which tend to form rows parallel to the ribs, and was thought to be caused either by certain winter feeds or by overheating.

Preliminary studies at the Department's Eastern Utilization Research and Development Division failed to support the prevailing theories and suggested that an external parasite was involved. With the close cooperation of the Animal Disease and Parasite Research Division it was established conclusively that the causative agent was the common sheep ked, Melophagus ovinus, known also as the sheep tick or louse fly.

Identification of the cause within two years was doubly fortunate since effective control measures against this parasite were already available. It seems feasible to eliminate the problem by implementing a suitable program of dipping or dusting with insecticide.

Mechanical Harvesting of Cherries. Mechanical harvesting of red tart cherries is now a solid commercial success, with 70% of the 1968 crop handled in this way. Jointly developed by ARS engineers and utilization research scientists and Michigan State researchers, the shake-and-catch system has produced 6-year savings on harvesting cost of nearly \$12 million and salvaged another \$15 million in fruit too sparse to allow traditional handpicking. Close cooperation among growers, processors, fieldmen and researchers has overcome substantial problems in quality losses and processing slow-downs. Acceptance of the new methods has been greatly accelerated by critical farm labor shortages; lessons learned during the development of systems for mechanically harvesting and processing tart cherries will be invaluable as the job of applying mechanized methods to harvesting the more difficult fruit crops goes on.

Market Potential Established for Vacuum Foam-Dried Whole Milk. The prospects for the commercial success of the beverage quality vacuum foam-dried whole milk developed by Department engineers were increased by the results from the market testing of the product in nine supermarkets in the Philadelphia suburbs. The product was priced at 4 cents less per quart than fresh whole milk sold in half-gallon containers out of the same refrigerated dairy case. Cost estimates indicate that this price differential is feasible when the dry milk is prepared from manufacturing (Class II) milk.



In a 12-week period, approximately 280 cases of 24-quart equivalent packages were sold--a very high rate of average sale per store for any new item. A substantial number of the purchases was made to supplement the customers' fresh milk supply rather than to replace it. The test product established a high consumer acceptance. Most users who were interviewed made very favorable comments about its taste, ease of storage, and reconstitution. Another sign of the strong acceptance of the product was in the repurchase pattern. More than three-fourths of the users said that they planned to repurchase the product, and two weeks later more than one-third had actually repurchased it.

Market Testing of EUDA Cheese. EUDA cheese, a new skim milk semisoft cheese developed several years ago by Department scientists has been successfully test-marketed in four Washington, D. C. suburban supermarkets. Cheese used in the test was produced during pilot plant scale production studies at the Department's Beltsville laboratories. Results of the twelve (12) week market test indicate that EUDA accounted for about eight(8) percent of total test-store cheese sales of other varieties included in the study while not significantly affecting the quantity of other varieties sold. EUDA could be profitably manufactured and sold by commercial cheese producers at prices significantly below those used during the test. Although the scope of the study did not permit critical evaluation of repeat purchasing, it was encouraging to find that 75 percent of EUDA buyers questioned by telephone indicated they planned to make additional purchases. Generally the results show that there is a definite place in the cheese market for EUDA.

Improved Separation and Composition Studies of Fats and Oils. Department chemists have developed methods for the preparation of derivatives and have improved procedures which substantially reduce the time required for the separation of fats and oils into constituent components. Accuracy is markedly improved over older methods and volatile components, important as odor imparting agents, are not lost. Samples of about one-ten-thousandth of an ounce may be used which makes the procedure useful for studies on living species. As a result of this investigation, the chemists have been called upon to direct collaborative studies involving industrial, academic, government and private research scientists leading to improvements in the state of the field and inter-laboratory agreement as to correct procedures and results in the study of fats and oils. The Codex Committee of the Food and Agriculture Organization-World Health Organization has expressed interest in these approaches with the aim of setting international standards for commercial trade in fats and oils. These studies are also of interest to industry since the quality and price of products are often dependent upon composition.

Improved Nutrition for Needy Families and School Children Made Possible Through Earlier Research by Department Engineers on Vitamin Fortification of Potato Flakes. Several 1965-1966 surveys showed that the nutrients most often lacking in families with good or poor diets were calcium, vitamin C and vitamin A. In continuing research on improvements in the potato flake process, USDA engineers had shown that fortification of potato flakes with

vitamins A, C, B<sub>2</sub> and niacin was practical and that vitamin retention on storage was excellent. This research is now paying off in improved nutrition for U. S. families, especially those in need, for vitamins A and C are now available in a low-cost food normally eaten in large quantity. In 1968, the U. S. Department of Agriculture purchased for its needy-family program over 15 million pounds of potato flakes fortified with vitamins A and C. Purchases in the first month of 1969 were over 2 million pounds. Schools also purchase quantities, amounts not known, for the school lunch program.

Ultraviolet Preservation of Maple Sap. Short-term preservation of maple sap is a practical, simple way to increase the output of a maple processing plant. Maple sap flows are erratic and can vary several fold in volume over a few days. When volume of incoming sap exceeds capacity of the processing plant, the previous choices were to make low-grade or sometimes unacceptable sirup from the stored sap or to dispose of "excess" sap. Chemical preservatives cannot be used to control spoilage because residues may appear in the sirup at up to 40 times the concentration used in the sap. Now, with ultraviolet pasteurization of incoming sap, combined with UV irradiation and mixing during storage, sap can be held up to six days and still make the same quality sirup as when fresh. Without the UV treatment, sirup quality is at least two grades poorer.

Evaluation of several methods for using UV in sap preservation was carried out in a research contract with J. L. Sipple & Son, a New York State maple sirup producer. The ability to hold sap for several days without sacrifice of quality is a necessity for efficient operation of the central evaporating plant, where the sap from many producers is processed. "Sap farming" is a way for farmers to get extra cash income during the slack early spring season without the technical knowledge or relatively large investment required for sirup production. Such gross income can average \$270 per season for a sap farmer with 500 tapholes, or 43% of his investment for equipment.

New Biodegradable Detergents From Animal Fats. In preparing detergents from tallow alcohols, the alcohol is sometimes treated with the chemical compound ethylene oxide in order to increase the solubility and to cause changes in the detergency of the final product. Department of Agriculture scientists have studied the use of organic oxides, containing 3 or 4 carbon atoms and related to ethylene oxide, to prepare biodegradable detergents. Results have shown that these detergents are more soluble and have better detergency than those obtained with ethylene oxide. These detergents, called ether alcohol sulfates, were completely degraded in river water and activated sludgesystems. They are attractive agents to be added to soaps since they disperse the "scum" caused by hard water.



## DAIRY UTILIZATION

## USDA and Cooperative Program

Problem Area		Scientist Man-Years		
		F.Y. 1969		
No.	Title and Activity	Intra-mural	Extra-mural	Total
410	New and improved meat, milk and egg products			
	Chemical composition and physical properties	24.7	0.3	25.0
	Flavor	6.8	0.8	7.6
	Color, texture and other quality factors	4.5	0	4.5
	Microbiology and toxicology	1.7	0	1.7
	Technology - process and product development	10.5	0.1	10.6
	Subtotal	48.2	1.2	49.4
601	Expansion of foreign markets for U.S. farm products			
	Technology - process and product development	2.8	0	2.8
	Subtotal	2.8	0	2.8
702	Protect food supplies from harmful microorganisms and naturally occurring toxins			
	Color, texture and other quality factors	6.0	0	6.0
	Microbiology and toxicology	1.4	0	1.4
	Subtotal	7.4	0	7.4
901	Alleviate soil, water, and air pollution			
	Technology - process and product development	0.9	0	0.9
	Subtotal	0.9	0	0.9
	TOTAL	59.3	1.2	60.5

Domestic program is supplemented by PL-480 funds in 6 countries totaling 941,379 U. S. dollars equivalent (Austria, Finland, India, Israel, Spain and Sweden).

## Problems and Objectives

The milk industry in the U.S. is characterized by a surplus of butter, non-fat milk, and cheese whey. Excess butter and nonfat milk are purchased by the Government for school lunch, welfare, and foreign distribution programs. Cheese whey is a serious stream pollution problem. Research on utilization of milk emphasizes the development of better processing procedures, improved dairy products, new and increased food uses for those milk components in excess supply, and more economical disposal of dairy wastes for which there is no profitable use.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Develop dry whole and concentrated milks of beverage quality.
2. Prevent the formation of or eliminate objectionable flavors in dairy products, and stabilize and intensify desirable flavors.
3. Prevent or eliminate contamination of dairy products with disease-producing microorganisms.
4. Develop food uses and more economical disposal processes for cheese whey.
5. Develop new cheese and milk fat products for food use.

## Progress - USDA and Cooperative Programs

### RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

#### A. Chemical Composition and Physical Properties

1. Milk Protein Composition and its Relation to Properties of Concentrated and Dry Milks. Application of new techniques has shown that  $\gamma$ -casein as formerly prepared is a mixture of proteins. A close relationship in the genetic control of  $\gamma$ - and  $\beta$ -caseins has been demonstrated. The observation that udder inflammation is apparently accompanied by changes in the distribution level of certain proteins indicates the possibility of developing a new test for mastitis.

An interrelationship between  $\kappa$ -casein and  $\alpha_s$ -casein in stabilizing the proteins in milk was demonstrated.  $\kappa$ -Casein loses its ability to prevent precipitation by calcium ions of small proportions of  $\alpha_s$ -casein when heated in a system containing salts and reducing agents. Increasing the ratio of  $\alpha_s$ -casein to  $\kappa$ -casein prevents the deleterious effect of heat; 0.5:1 gives partial protection, 1:1 gives complete protection (100°C. 5 minutes). Selective chemical modification of reactive amino acids in the caseins were

continued in an attempt to understand the interaction between  $\kappa$ -casein and  $\alpha_s$ -casein and how this interaction contributes to the stability of the casein micelle. The amino acid sequence of part of the peptide which differentiates  $\alpha_s$ -casein B from  $\alpha_s$ -casein A has been identified. The interactions of bovine serum albumin with solvent compounds in water-organic solvent mixtures were compared with those obtained for  $\beta$ -lactoglobulin and similarities and differences interpreted in terms of protein structure.

Swine and cow caseins differ considerably in composition. The principal swine caseins were isolated and found to be interchangeable with corresponding bovine caseins in the  $\alpha_s$ - or  $\beta$ -casein complexes with  $\kappa$ -casein and calcium ion.

Electron microscopic examination of the effects of heat on milk showed whey protein fraction precipitates and unorganized structures quite similar to the bridging material found in gelled milks. Size increase of micelles from evaporated milks is hypothesized to result from deposition of soluble casein, whey proteins and ionic constituents on the micelle surface. The effect of incremental addition of polyphosphate (0.01 to 0.1%) to 3:1 evaporated milk showed no apparent effect on micelle structure, but an increase of soluble casein was noted by solvation experiments.

A size distribution counting technique and electron microscopy allow a comparison to be made of the fat globule size in normal and spontaneously rancid milks. The latter may result from a reduction in size, and consequent increase in surface area, of the fat globules. Free and bound lipids have been found in a variety of casein preparations and fractions. The compositions are complex.

From acid-base titration data, obtained over a wide pH range, it is concluded that there is only slight alteration in molecular form or structure as the whey protein,  $\beta$ -lactoglobulin, passes from dilute solution to the crystalline state. In structural studies conducted at the University of Graz, Austria, under a PL-480 grant, the elongation of the deoxyribonucleic acid molecule after addition of acridine was confirmed. It appears from small-angle X-ray data that every acridine molecule bound results in detwisting at the bonding site.

Unnatural nucleic acids and their relation to milk composition are being studied in PL-480-sponsored research at Israel Institute of Technology, Haifa. Results indicate that 5-fluorouracil inhibits selectively the synthesis of certain phage-induced proteins. The inhibition appears to occur as a result of selective action on messenger ribonucleic acid synthesis.

In research at EU efforts are being made to isolate bovine milk lysozyme. Lysozyme is a major protein in human milk; quite minor in cow's milk, more plentiful in cow colostrum. It may be important in the diet; it is not destroyed in the intestinal tract. It was found that colostrum contained approximately three times the enzyme activity of normal milk. Using colostrum as a source of lysozyme, purification methods now available have not yielded the desired homogeneity for physical and chemical studies.

The B protein,  $\alpha$ -lactalbumin, of lactose synthetase has been isolated from cow, swine, goat, sheep, human, buffalo, and guinea pig milk. The proteins appear to be homogeneous on electrophoresis, but the swine preparation yielded two bands on double diffusion against antiserum for the protein. Further experimentation has revealed two bands for the sheep, goat, human and buffalo  $\alpha$ -lactalbumins. This research is proceeding under a grant at Oklahoma State University.

2. Effect of Feed on Milk Fat Composition. Contract research at the University of Maryland (now completed) indicates that if one desires a high degree of unsaturation in milk fat, the cow should be maintained on a high forage (corn silage) ration that is ground and pelleted. When the pellets were fed as the only source of forage, the level of fat in the milk was lower and the fat contained more unsaturated fatty acids than was obtained from cows on a ration containing some grain.

In PL-480-supported research at the Biochemical Institute, Helsinki, Finland, more than 8800 pounds of standard milk per year have been produced by animals on the protein-free purified feed with urea as the sole source of nitrogen. Supplementing the synthetic diet with natural feed of low protein content, urea remaining the major source of nitrogen, increases milk production considerably. By using certain feeding combinations, milk poor in fat but rich in protein can be produced. No differences have been observed in the activities of enzymes in milk produced on normal feed with that produced on the synthetic diet. The cholesterol content of the blood, normally low in the animals on a synthetic diet, is raised significantly upon the slight addition of normal feed to the animals' diet.

These studies provide basic information needed for the development of improved milk products.

## B. Flavor

1. Control of Oxidative Flavors. Ozone in the air used for spray drying milk will produce serious off-flavor in the finished product. The compound responsible for the off-flavor has been identified as 6-trans-nonenal, which has a threshold of less than 0.07 parts per billion in fresh whole milk. This compound is considered to originate by trace ozonolysis of minor lipid components on the surface of the dried product. The concentrations of ozone producing this effect are in the range of those found in the earth's atmosphere polluted with auto exhaust fumes during the summer months. Atmospheric ozone can be removed by placing activated charcoal filters in the dryer's air inlet. This modification allows year-round production of high quality powders.

In contract research at the University of Maryland, a linear relationship was observed between the quantity of tocopherol acetate added to the ration and the tocopherol content of the milk produced. The natural tocopherol content of various forages, the effect of growth stage at harvesting and method of preserving on quantity of natural tocopherol available to the cow, and the



level of additional  $\alpha$ -tocopherol required to render milk, produced on dry feeds, resistant to oxidized flavor were determined.

2. Compounds Contributing to Flavor in Milk and Milk Products. Previous study had shown that processing milk to evaporated milk caused an increase in level of methyl ketones. Analyses of five commercial samples of stale evaporated milk showed that 29-37% of the total methyl ketone potential of these samples were present as free methyl ketones. Taste panel studies revealed that free methyl ketones were present in sufficient concentration in only one of these samples to contribute to off-flavors of the product. Accordingly, methyl ketones are not considered of major significance in the stale off-flavor of evaporated milks.

In contract research at Oregon State University, a large taste panel has been used in conjunction with advanced chemical techniques to determine the contribution of various butter constituents to desirable flavor. Greater than 90% of the volatiles present in fresh, sweet cream butter in excess of one part per billion, and several below this level, have been identified. The most significant contributors to butter flavor were found to be butyric acid, caproic acid, diacetyl, dimethyl sulfide, acetaldehyde and delta-decalactone. Compounds of less significance include aldehydes, methyl ketones and other lactones. Synthetic butter flavor blends prepared were an improvement over commercial butter flavor concentrates but not an exact duplication of natural butter flavor. Taste panel studies revealed that college students prefer good quality sweet cream butter to all brands of margarine tested.

The products of a browning-type lactose-casein interaction are being isolated and identified to establish the contribution, or non-contribution, of the skim milk portion to the off-flavor of stored dairy products.

New micromethods were developed to analyze butterfat for such compounds as 1-monoglycerides,  $\alpha$ -glyceryl ethers, and secondary alcohols.

In contract research at Pennsylvania State University, the delta oxidation of saturated fatty acids in the mammary gland is proposed as the mechanism for formation of lactone precursors in milk. The content of hydroxy triglycerides and  $\alpha$ ,  $\beta$ -diglycerides in milk was determined.

The basic flavor studies provide information needed for the development of improved milk products.

### C. Color, Texture and Other Quality Factors

Fortification of pasteurized whole milk with iron showed that iron in the amount of 10 mg. per quart could be added without objectionable off-flavor development. The iron was added preferably as solutions of either ferric ammonium citrate or ferric choline citrate.

The extent of hydrolysis of polyphosphates in sterilized milk products is of concern regarding acceptance of these compounds as stabilizers. Data



obtained in developing assay procedures showed that the polyphosphates are hydrolyzed at a pH below 5.0. A procedure for minimizing hydrolysis was worked out.

The distribution of antioxidants, such as the gallic acid esters, in milk or model systems simulating milk is influenced both by partition effects and binding by proteins. As the length of the carbon chain in a homologous series of gallates increases, both the concentration in oil/concentration in water partition coefficient and amount bound by milk proteins increase. After a consideration of this partition and binding data, whole milk powders containing mixtures of antioxidants giving theoretical optimum distribution in all phases of the product were made. These powders are now undergoing storage stability tests.

Peptides containing different molar ratios of amino acids and sugars are released from milk proteins coagulated by enzymes, heat and chemical additives. A previously unreported glycomacropeptide containing aromatic amino acids released from calcium caseinate by rennin has been isolated and characterized. A crude preparation of a milk whey component which releases a kinin-like peptide upon trypsin digestion has been made. The pharmacological action of this peptide on smooth muscle is suppressed by the addition of food grade antioxidants, the first known demonstration of interference of biological activity by antioxidants of the type used for food preservation.

#### D. Microbiology and Toxicology

Reduced nicotinamide adenine dinucleotide oxidase enzyme from Bacillus cereus T spores, previously thought to be a single enzyme, has been resolved into two components. Several physical and biochemical properties of the components have been compared and found to be substantially different, with the major component being unique to spores. A new enzyme not previously detected in spores, disulfide reductase, has been discovered in three spore species. The enzyme from Bacillus megaterium spores has been partially purified and a number of its properties determined. The uniqueness of spore enzymes could provide a basis for explaining the resistance of microbial spores to thermal destruction.

In PL-480-sponsored research at N. P. Agricultural University, Pantnagar, India, synthetic media have been developed to allow analysis of specific dipicolinic acid (DPA) synthesis inhibitors to be tested in a defined system. Picolinamide and nicotinamide appear to be DPA synthesis inhibitors, allowing sporulation with production of heat-sensitive spores.

Microbiological research related to cheese flavor development is in progress at National Dairy Research Institute, Karnal, Punjab, India, under a PL-480 grant. Forty-three cultures of lactic streptococci have been isolated from milk and milk products in the area and characterized biochemically. Seventeen selected starter cultures were examined for rate of acid production, total and volatile acid production, formation of flavor compounds and protein breakdown. The protein degrading ability noted with a culture of Leuconostoc

citronocum is possibly significant from the standpoint of cheese ripening.

These studies provide basic information needed for the development of improved sterile milk and cheese products.

#### E. Technology - Process and Product Development

1. Dry Whole Milks. A market test was held in 9 supermarkets in the Lansdale, Pa., area during a 12-week period. The vacuum foam-dried whole milk labeled, "Dairy Fresh," was sold in 12-ounce cans containing the equivalent of 1 quart of fresh whole milk. Dairy Fresh was priced 4¢ per quart less than fresh whole milk in 1/2-gallon containers in the same store. In-store demonstrations, carried out during the first 3 days of the test, were the only form of advertising used. After the second week a stable market of about 2 cases per store per week was established. The test product established a high consumer acceptance. Most users who were interviewed made very favorable comments about its taste, ease of storage and reconstitution. A detailed cost engineering evaluation of the process based on a plant containing two commercial-sized driers processing Class II milk predicted that the process would be commercially feasible. There is some interest in operating the process commercially. Reconstituted vacuum foam-dried whole milk can be used in any application in which fresh milk is used. In addition, when reconstituted to higher solids than fresh milk, it can be used for whipped topping, coffee creamers, cereals and other uses where a richer product is desired.

In conjunction with the foam-spray drying process, pilot-scale equipment for cooling and packaging dry whole milk in an inert atmosphere immediately after drying was designed, developed and tested. Oxygen levels of less than 0.01% in the head space gas of the finished package were achieved. Liquid inert gas was used to cool the powder and protect it against oxygen absorption in the conveying system. The powder was transferred, without exposure to air, and packaged under an inert atmosphere.

Research data indicates that stable powders, reconstitutable to beverages, having essentially the same nutrients and flavor as whole milk, can be reconstructed from nonfat milk solids, processed butteroil and naturally-occurring milk flavoring agents.

It was also found that wet casein precipitated from iron fortified skim milk could be homogenized back into milk and spray-dried to produce a powder reconstitutable to a high protein, high iron containing beverage having the flavor of whole milk.

2. Skim Milk and Whey Powders. Experiments designed to determine the effects of temperature and humidity on the free flowability of milk and whey powders were completed. Lactose crystallization was implicated as the primary cause of powder caking. Observed differences between the powders examined were interpreted in terms of the surface disposition of the individual milk constituents on the various powders and the mechanism of water sorption

established in this research. Additional studies indicate that drying procedures based on direct water vapor transfer from aqueous systems such as spray drying or lyophilization may have a profound effect on casein micelle organization.

Several runs were made in commercial plants in which foam-spray drying was attempted using the DPL's liquefied carbon dioxide injection process. Of three types of commercial dryers, some runs were good; others poor. Skim milk dried better than whey. The main problem has been coating and sticking of the dried product in out-going surfaces of the dryers. The proposed solution is to provide an optimum balance between injected gas and other operating conditions of the dryer.

In contract research at Michigan State University, equipment designed to measure rate of heat transfer through beds of milk powder was field-tested. A study of twelve different dry milk manufacturing plants showed that the temperature of the dry milk at the sifter varied from a high of 128°F. to a low of 87°F. in the various plants. Most plants used room air injected into the powder stream for cooling. Heat transfer in masses of hot powder was very slow; dry milk in 50- and 100-pound packages cooled only 8°F. per day, in 3000-pound bins only 2°F. per day, when stored at room temperature. Without rapid cooling, loss in quality occurs.

Leads developed and pursued during research on dehydrated, reconstructed milk led to the development of a skim milk powder having excellent whipping properties. The new product is obtained by subjecting skim milk to homogenization prior to conventional spray drying. Large batches of this material have been made during the report period to meet demands for samples.

3. Whey Products. Sweet cheese whey can be converted into a low-fat spread by homogenizing butteroil into it and reducing water content to 20%. Production parameters and use of this material are being investigated. The use of whey concentrate, combined with starch, cream and cocoa flavoring, showed promise as a sterilized pudding-type product.

4. Cheese. Excellent quality low-fat cheese has been consistently manufactured on a pilot plant scale. The cheese was cured for 6-12 weeks, packed in consumer-sized packages, and dispensed in four stores of a local food chain through a cooperative project with the Economic Research Service. Results indicate that this type of cheese was accepted and is marketable. Complete details of the study have been published by ERS.

5. Anhydrous Milk Fats. The feasibility of producing heat-treated (ghee-like) anhydrous milk fat in a continuous process was demonstrated. The product had good storage stability at room temperature for periods of six months or longer in the absence of oxygen or light. It is being tested as a source of fat and flavor in bakery products such as cakes and bread. Additionally, a quantity of this fat has been used for pilot plant scale experiments in combination with coconut fat to produce new butter cream candies.



Butteroil heated in the presence of cocoa mix gave a product that was more intense in odor and flavor than high temperature ghee. Low fat spreads, with good flavor character and physical consistency, were prepared with ghee-type oils and with as much as 50 percent water. Butteroil and ghee were fractionated by zone precipitation and fractional crystallization methods.

#### RPA 601 - EXPANSION OF FOREIGN MARKETS FOR U. S. FARM PRODUCTS

- A. Chemical Composition and Physical Properties (no current research)
- B. Flavor (no current research)
- C. Color, Texture and Other Quality Factors (no current research)
- D. Microbiology and Toxicology (no current research)
- E. Technology - Process and Product Development

Sweet and acid cheese wheys can be combined with soy flour, flavored, condensed and dried to produce powders which reconstitute to high protein beverages simulating milk in nutritional value. Suspension stability is enhanced by homogenization and soybean flavor is reduced by condensing and spray drying. Objectionable soybean flavors are steam distillable. The acceptance of a simple mixture of sweet Cheddar whey and soy flour has been tested informally by UNICEF using malnourished children in Ethiopia, India and Peru. Unofficial results from conducted experiments in Ethiopia and India indicated good acceptance, but gastrointestinal disturbance accompanied high intake of mixture. The investigator conducting the Peru experiments considered the mixture a superior child feeding formula if properly used.

#### RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

- A. Chemical Composition, Physical Properties and Structure (no current research)
- B. Flavor (no current research)
- C. Color, Texture and Other Quality Factors

1. Milk Allergy. Serological studies on fractions of milk proteins, such as  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin, show that the preparations available are mixtures of two or more proteins. Likewise, the availability of class-specific antisera has made it possible to confirm the heterogeneity of immunoglobulin fractions as typically prepared.

Low molecular weight antigens were generated by brief pepsin hydrolysis of bovine serum albumin,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. This finding may explain why the individual giving a negative skin test shows an allergic response on ingestion and enzymatic hydrolysis of the food material being tested. Hydrolyzed casein was deficient in the property of producing a new antigen.

In PL-480-sponsored research at Hadassah Medical School, Jerusalem, Israel, patients with gastrointestinal sensitivity to milk were challenged with different milk components and showed pathological reactions to various proteins, with  $\beta$ -lactoglobulin being involved more than other antigens. Most processed milk preparations also elicited allergic reactions in these patients, although experiments with animals showed that the processing of milk affects its antigenicity to various degrees. The only processing that yielded allergen-free product was that of protein breakdown (hydrolysis); heating and removal of antigens were found to be insufficient for obtaining non-allergenic preparations.

#### D. Microbiology and Toxicology

1. Salmonella in Cheese. Factors affecting the growth and survival of Salmonellae in artificially contaminated Cheddar and Colby cheese were studied. Manufacturing variables tested included milk pasteurization, size of starter inoculum, titratable acidity and cheese pH, types of lactic culture, chemical additives, salt, moisture, supplemental cheese microorganisms and curing temperatures. Salmonellae survival in the cheeses varied from two to seven months. The rate and amount of acid produced during making, the pH of the cheese and the type of lactic starter had the greatest effect in suppressing the growth of Salmonellae. Pasteurization of the milk, salt, moisture and chemical additives had little or no effect on the Salmonellae. The addition of large numbers of Propionibacterium and Leuconostoc seemed to favor the survival of Salmonellae. Lactobacilli and enterococci tested had no effect.

This study provides basic information regarding survival of Salmonellae in cheese and emphasizes the need for proper sanitation and control in the manufacture of cheese.

#### E. Technology - Process and Product Development (no current research)

##### RPA 901 - ALLEVIATE SOIL, WATER, AND AIR POLLUTION

#### A. Chemical Composition, Physical Properties and Structure (no current research)

#### B. Flavor (no current research)

#### C. Color, Texture and Other Quality Factors (no current research)

#### D. Microbiology and Toxicology (no current research)



## E. Technology - Process and Product Development

1. Concentration of Dairy Products by Reverse Osmosis. In research to make whey disposal less costly and its utilization more economical, extensive pilot plant studies on conditions affecting concentration of whey by reverse osmosis were conducted. Optimum operating conditions, as indicated in laboratory trials, were tested for continuous 5- and 8-hour periods, using up to 150-gallon lots of whey. Variables included pressure, feed rate, pH, temperature, and membrane porosity. Concentration of up to 35% total solids was obtained at 800 psi with a retention of 96 to 99% of the solids, depending upon the membrane porosity. A concentration rate curve was established which can be "scaled up" to accurately predict the unit capacity required to process a given volume to a desired level of solids.

## Publications - USDA and Cooperative Programs

## RPA 410 - NEW AND IMPROVED MEAT, MILK, AND EGG PRODUCTS

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#### RPA 601 - EXPANSION OF FOREIGN MARKETS FOR U. S. FARM PRODUCTS

##### Technology - Process and Product Development

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#### RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

##### Color, Texture and Other Quality Factors

Spies, J. R. Determination of tryptophan in corn (Zea mays). Agr. Food Chem., 16, (1968), 514-516.

##### Microbiology and Toxicology

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## MEAT UTILIZATION

## USDA and Cooperative Program

Problem Area		Scientist Man-Years		
		F.Y. 1969		
No.	Title and Activity	Intra-mural	Extra-mural	Total
410	New and improved meat, milk, and egg products			
	Chemical composition and physical properties	2.5	0	2.4
	Flavor	4.7	0.4	5.1
	Color, texture and other quality factors	4.9	0	4.9
	Microbiology and toxicology	1.4	0	1.4
	Technology - process and product development	0	1.0	1.0
	Subtotal	13.5	1.4	14.9
702	Protect food supplies from harmful micro-organisms and naturally occurring toxins			
	Microbiology and toxicology	3.0	0.5	3.5
	Subtotal	3.0	0.5	3.5
907	Improved income opportunities in rural communities			
	Chemical composition and physical properties	1.3	0	1.3
	Technology - process and product development	2.3	0.2	2.5
	Subtotal	3.6	0.2	3.8
	TOTAL	20.1	2.1	22.2

Domestic program is supplemented by PL-480 funds in four countries totaling 259,572 U. S. dollars equivalent (Austria, The Netherlands, Poland and United Kingdom).

## Problems and Objectives

Shelf life of fresh meat, currently about 5 days, needs to be lengthened so that more efficient processing and packaging in centralized locations with resultant economies is possible. Processed meat products, two-thirds of our pork and significant quantities of other meats, need better keeping quality relative to color and flavor retention in distribution channels. Technical advances are needed to enable the numerous small meat processors to manufacture better products. Contamination of meat with pathogenic microorganisms is a serious problem which must be eliminated through improved processing techniques.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Prolong the retail shelf life of fresh meat to 15 to 20 days.
2. Provide procedures for the preparation of meat products with initially desirable organoleptic and physical characteristics which are retained in storage.
3. Provide technology which can be applied to the problems of small-scale processors and improve the meat products of rural industry.
4. To improve meat processing methods so that the consumer can be assured of safety in meat and its products.

## Progress - USDA and Cooperative Programs

### RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

#### A. Chemical Composition and Physical Properties

Various methods for the preparation of myosin were evaluated with respect to the effect of conditions during preparation on the yield of the protein and on its enzymatic activity; one of these was adopted because of its distinct superiority. Myosin stored in glycerol or sucrose was shown, by ultracentrifugal analysis, to be free of derivatives which form rapidly when the protein is stored in the absence of such polyhydroxy compounds. Various contaminants in myosin preparations were removed by chromatography, including one tentatively identified as nucleic acid, and the specific enzymatic activity of the myosin was substantially increased. Binding of nucleic acid to the resin was found to be very tight, which led to an investigation of a simple batch operation to replace the column method. Increased purity was accompanied by increased susceptibility of the enzyme to inactivation at a mildly alkaline pH. A rapid and efficient method was developed for chromatography of the substrate adenosine triphosphate (ATP) with a view to application for both purification and analytical purposes. Various supporting analytical techniques have been or are being developed. These include gel

electrofocusing, the preparation of  $\gamma$ -P<sup>32</sup>-ATP, a continuous automated ATP-ase assay, a modified biuret method of protein assay, and ultracentrifugal analysis of myosin in a density gradient.

These studies provide basic information needed for the development of improved meat products. Myosin is the most important of the meat fibrillar proteins. A more complete understanding of its activity as enzyme and as a polymer is necessary to solving the meat tenderness problem and many problems of binding in meat products.

## B. Flavor

1. Meat. Investigation of the development of flavor in cured, smoked ham involves chemical and sensory evaluation of cured and uncured raw, cooked, and smoked hams. The curing salts, particularly sodium nitrite, modify the meat components to give the characteristic cured flavor; smoke flavor is more noticeable with cured meat. A basic, water-soluble, meaty aroma is present in all pork; the smoke and cured flavor are in the lipid phase and can be separated from the basic meaty flavor. Thus, cured and smoky aromas are distinct and do not modify the precursors of the basic aroma. Some variations among the profiles of the variously treated hams are observed, but no single component smells like raw, cured or smoked ham. Light and dark pork muscles were analyzed for lipid composition. In the light muscle with significantly higher lipid levels, the triglyceride fraction (approximately 80% triglycerides and 20% phospholipids) was similar in fatty acid composition to the triglycerides of dark muscle (60-70% triglycerides, 26-37% phospholipids). The phospholipids of dark muscle were higher in polyunsaturated and lower in monounsaturated fatty acid.

Two unusual compounds, 2,4,5-trimethyl- $\Delta^3$ -oxazoline and 3,5-dimethyl-1,3,4-trithiolane, were isolated and identified from the fractions possessing characteristic meat aroma of boiled beef. A cooked, freeze-dried sample was analyzed after 18 months of storage. Results indicated that flavor deterioration was not due to formation of new flavor compounds. There was a decrease in some types of compounds and increase in other classes. Meaty flavor fractions decreased in quantity. Freeze-dried beef which was stable one year under N<sub>2</sub> at 60°F. developed an off-odor when stored at 60°F. in air. The volatile flavor fraction was a complex mixture of over 35 compounds.

2. Smoke. Studies on the chemical composition of wood smoke were continued with the additional identification of 12 components from an ether extract of a commercial smoke solution not previously identified in wood smoke preparation. A novel technique was developed to concentrate smoke components so sensory evaluations could be carried out on fractions separated by gas chromatography. The composition of one fraction, designated as smoky by the taste panel, is being studied and preparations based on its composition are under investigation. An assay procedure to determine the carcinogenic polynuclear hydrocarbon benzo(a)pyrene has been established and collaborative studies with Food and Drug Administration carried out.

These basic studies regarding flavor are providing information needed for the development of improved meat products.

### C. Color, Texture and Other Quality Factors

Research on the isolation of heme pigments from heart extracts has led to the development of a continuous chromatographic apparatus, the definition of the optimal and necessary operating conditions for preparing and operating the column and the invention of devices and development of techniques required to attain these operating conditions. The apparatus has been proved out in purifying myoglobin, the pigment of muscle tissue, and is in routine use in the laboratory. The apparatus has also been proven useful for the separation of milk proteins from lactose, salt, and riboflavin of skim milk, a technique which has some commercial significance. The reactions of hydrogen peroxide with heme pigments, the most important pathway of pigment deterioration during meat storage, were investigated. The reactions produce two pigments, a green and a red pigment under acid and alkaline conditions, respectively. Studies of the mechanisms of the reactions showed them to be complex, involving (1) inactivation of peroxide by binding, (2) different reaction mechanisms depending on peroxide concentration, and (3) production of spectrally identical but chemically different intermediates.

A new and improved reflectance method of measurement of meat pigment (MbO, Mb<sup>++</sup>, Mb<sup>+++</sup>) proportions was devised during research under a grant at Florida State University, Tallahassee. Methods were developed for determination of metmyoglobin reducing activity (MRA). The effects on MRA of beef muscle of pH, sodium chloride, niacin adenine dinucleotide, succinate, glutamic acid, intermediates of glycolytic pathway, glycerophosphate, dinucleotides and dicumarol were examined. Basic information was obtained in model system studies of the effect of heme catalysts on autoxidation of methyl linoleate and linolenate.

The important flavor compounds alk-2,4-dienals are formed spontaneously in oxidizing lard in negligible quantities. Data supported the theory that scission of linoleate hydroperoxide isomers normally occurs at the carbon linkage between a double bond and the hydroperoxide group. This scission mechanism is changed by heat or Cu<sup>++</sup> ions with formation of the dienal. A similar mechanism appeared to exist for oxidized triolein, but the hydroperoxide isomers probably were not present in equal quantities. Basic information on meat lipid autoxidation was gained in preliminary studies of the effect of sodium chloride, phosphate buffer, metals, chelators, chelates, and pH on sodium carbomethoxy cellulose-lard model system autoxidation.

These studies provide basic information needed for the development of improved meat products.

### D. Microbiology and Toxicology

The ability of certain microorganisms to decrease the peroxide value (PV) of rancid lard and to prevent increases in the PV in fresh lard emulsions has



been extended to a model system containing lard, methyl cellulose, and cells in a dried mixture. When held in the dark at 25°C., the whole cells of several bacterial species prevented increases in PV whereas the uninoculated controls developed a PV of approximately 100. At 20°C. lard-cell powders exposed to light were not protected by the cells, thus the microbial antioxidant was possibly photolabile. The antioxidant activity of cellular extracts obtained with various solvents indicates that the activity is due to non-enzymatic agents.

In PL-480-supported research at the University of Veterinary Medicine, Vienna, Austria, small amounts of sodium lauryl sulfate have been found effective in reducing the thermal resistance of Streptococcus faecium.

These basic microbiological studies are needed to aid in the improvement of fresh, cured, cultured and canned meat products.

#### E. Technology - Process and Product Development

1. New Smoked Meat Products. The terminated contract research at Michigan State University, East Lansing, dealt with the chemical-organoleptic aspects of meat smoking, smoke processing variables and their effects, and the development of new smoked products. Smoke flavor was shown to depend on the penetration of phenols, while cured flavor was shown to develop independently, owing to the interaction of the curing agent, sodium nitrite, with meat. Analyses indicated that smoke constituents are relatively concentrated near the surfaces of smoked meats with sharply decreasing penetrations occurring toward interiors. Using several criteria, smoke was shown to interact with meat proteins. The type of sausage casing and humidity can be selected and particulate-free smoke used to improve the smoking operation. Several promising new smoked meat products were developed, including a smoked pork sausage and a ham base flavoring (for ham loaves) from pork trimmings, a smoked corn beef, and smoked lamb breast.

2. New Frozen Meat Products. Semi-roasted, frozen meats were found to be extremely stable and offer excellent possibilities for commercial adoption. Various chemical changes during freezer storage were observed to be closely correlated with flavor changes. The most stable cooked and frozen meat dishes are those incorporating mixtures of starches or other polysaccharides. Modified starches, such as crossbonded waxy milo, can prevent the textural failure during freezer storage of gravies and sauces used in cooked dishes. The research, now completed, was conducted under contract at the University of Missouri, Columbia, Missouri.

3. Cooking Processes. Under contract research at Cornell University, Ithaca, N. Y., investigations to improve meat processing and the institutional cookery of meat were concerned with thermal properties, chemical responses to heat including the principles of low-temperature cookery, the composition of institutional size cuts, and variations in the composition of connective tissues in meat derived from animals of different age and sex. The composition of beef ribs marbled at different levels was determined for



the value of the data as such and for use in relating composition and thermal conductivity. It was observed that, as marbling decreased, percentages of muscle, bone and tendon increased and seam fat decreased. The mucopolysaccharides of connective tissue were shown to be more abundant in beef from younger animals; in all samples the principal chondroitin sulfate was the B variety. The superior tenderizing effect of low-temperature cooking was shown to arise from persisting and greater activity of collagenolytic and proteolytic enzymes during such cooking, and the water-holding capacity was higher, appearing to have a favorable effect.

4. New Lamb and Mutton Products. Smoked lamb sausage surpassed smoked pork sausage in acceptability tests, with three of the five chosen formulas being rated in the 50 (about average acceptable) to 60 (highly acceptable) range. Experimental lamb sausages were smoked (1 hr.) to a chestnut brown color by burning hickory sawdust. All experimental sausages using lamb in combination with pork and beef outranked available fresh pork sausage in acceptability by the panel. These products are being prepared and evaluated under contract research at Pennsylvania State University, University Park, Pa.

#### RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

A. Chemical Composition and Physical Properties (no current research)

B. Flavor (no current research)

C. Color, Texture and Other Quality Factors (no current research)

D. Microbiology and Toxicology

1. Eliminating Salmonellae from Meat. The premise that psychrophilic spoilage types of bacteria will overwhelm pathogenic types does not hold for Salmonellae in meats. Studies on the growth and survival of Salmonellae in fresh ground pork have shown that they would grow at 10°C. in competition with the background flora, even when the Salmonellae constituted less than 5% of the initial flora. It was also found that levels of salt, nitrate and nitrite that are tolerated in meats are insufficient to have any influence on the growth of Salmonellae. Although they would not grow at 4°C., they survived for long periods at this temperature. It was clearly shown that whereas decreasing temperatures increase the inhibitory effects of pH and NaCl, they decrease the lethal effects.

Studies on the mode of action of selenite-cystine broth in enhancing the isolation of Salmonellae has led to the development of a new procedure that may shorten the isolation procedure for Salmonellae from dried foods that are frozen or subjected to other treatment that damages the cells. Aliquants of the sample are placed in a basal medium and shaken for 4 hours at 37°C., selenite and cystine are added, and the shaking continued for an additional 18 hours. With samples examined to date, the recovery of Salmonellae is almost as good as with lactose pre-enrichment, and 24 hours less time is

required. In studying the mode of action of tetrathionate broth, another selective medium for Salmonellae, 30 Salmonellae and several other enterobacteria have been examined for tetrathionate reductase (TTR) activity. All cultures produced TTR, but the induction period for TTR activity was much less among the Salmonellae.

2. Production of Enterotoxin by Staphylococci. The search for a genetic link between the ability of Staphylococcus aureus to produce Enterotoxin B and some other physiological characteristic of the cell has continued. The enterotoxin has been shown to be stable in brain heart infusion broth for more than a month when stored at 5°C. One strain of S. aureus, no. 243, was found to consistently segregate into two differently pigmented colonies. Although the gold-pigmented colonies produced less toxin than the "off-white" ones, the decreased toxin production may have been related to decreased growth.

Assay of large numbers of strains of Staphylococcus for Enterotoxin B production is practical only by demonstration of a toxin-antitoxin reaction directly in petri dishes on which the colonies grow initially. Relatively large quantities of antiserum are required, however. Burro antiserum, furnished by Fort Detrick, was unsatisfactory for use in plate assays for enterotoxin, but rabbit antiserum from the University of Wisconsin appears to be satisfactory.

3. Utilizing Microbial Antagonisms. In contract research at the University of California (Davis), radiation-killed cells of Pediococcus cerevisiae were found to be highly effective in producing low pH in meat products provided a small amount of carbohydrate was made available. This low pH, achieved without cell proliferation, was capable of inhibiting toxin production by both Clostridium botulinum A and staphylococci in stored meat products. The Pediococcus cells are irradiated at a level of 0.75 M rad. They can be lyophilized and stored for more than two months without losing their acid-producing capacity.

E. Technology - Process and Product Development (no current research)

#### RPA 907 - IMPROVING INCOME OPPORTUNITIES IN RURAL COMMUNITIES

A. Chemical Composition and Physical Properties

Investigations to develop improved rapid analytical methods for process and product control were concerned with evaluating existing rapid methods of determining the fat content of fresh and frozen meat materials and developing new techniques that increase accuracy and precision and reduce time, cost, convenience, and skill required in analyses. Application of the standard solvent extraction using ether or petroleum ether in Goldfish or Soxhlet extractors, when modified as to sample size, preparation, and drying prior to extraction, and duration of extraction, was studied. Results obtainable with several of the Babcock-type methods were investigated. A method involving enzymatic, heat, and mechanical degradation of samples, followed

by solvent extraction and determinations of fat in solvents using refractometry, has been found promising.

B. Flavor (no current research)

C. Color, Texture and Other Quality Factors (no current research)

D. Microbiology and Toxicology (no current research)

E. Technology - Process and Product Development

1. Ready-to-Eat Meat Products. Contract research (now completed) at Southern University, Baton Rouge, La., demonstrated the feasibility of using microwave ovens for barbecuing in small-scale commercial operations. Recipes and directions have been developed.

2. Meat Emulsions and Products. Research under a related work unit, now discontinued, established that the melting characteristics of fat raw materials and the temperatures attained when meats are comminuted to produce meat emulsions are factors affecting the stability of the emulsions. Lack of this stability is frequently a commercial problem. Part of this work involved the first determination of the melting characteristics of meat fatty tissues as such. The method used, differential thermal analysis, has since been further applied to obtain data on the effects of commercial handling on the fats. Results obtained early under the present project indicated that the shear force applied in producing meat emulsions is a strongly interacting factor affecting not only the stability of emulsions, but also important qualities of the frankfurters and bologna prepared on cooking the emulsions. As a consequence, a processing study was undertaken. The results indicate that the quality of meat emulsions and that of meat products prepared from them depends to a large extent on optimally relating the melting character of fats and temperatures, rates of temperature rise, and the shear applied during comminution.

## Publications - USDA and Cooperative Programs

## RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

Chemical Composition and Physical Properties

Mozersky, S. M., Pettinati, J. D. and Kolman, S. D. Micromethod for determination of orthophosphate in presence of adenosine triphosphate after deproteinization with trichloroacetate buffer. Anal. Chem., 40, (1968), 788-791.

Flavor

Chang, S. S., Hirai, C., Reddy, B. R., Herz, K. O. and Kato, A. Isolation and identification of 2,4,5-trimethyl-3-oxazoline and 3,5-dimethyl-1,2,4-trithiolane in the volatile flavour compounds of boiled beef. Chem. & Ind., (1968), 1639-1641.

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Color, Texture and Other Quality Factors

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## ANIMAL FATS AND OILS UTILIZATION

## USDA and Cooperative Program

Problem Area		: Scientist Man-Years		
		: F.Y. 1969		
No. :	Title and Activity	Intra- :	Extra- :	Total
:		mural :	mural :	
411 :	New and improved products from wool, hides,	:	:	:
:	skins and animal fats	:	:	:
:	Chemical composition, physical properties	:	:	:
:	and structure	6.2 :	0.7 :	6.9
:	Chemical and physical investigations to	:	:	:
:	improve products	30.0 :	1.3 :	31.3
:	Technology - process and product	:	:	:
:	development	2.7 :	0 :	2.7
:	TOTAL	38.9 :	2.0 :	40.9

Domestic program is supplemented by PL-480 funds in 3 countries totaling 153,607 U. S. dollars equivalent (France, India and Poland).

## Problems and Objectives

Finding outlets for inedible fats has been a problem ever since synthetic detergents began to replace soap. The problem has been complicated further by the increase of its production as a result of the continuing increase in meat consumption. Previous utilization research activities have been effective in providing markets for about 20 percent of the 4.5 billion pounds that is produced per year. In order to maintain current markets and provide for new and economical ones for this byproduct of the meat industry a strong and progressive research program is necessary. A promising area that is accounting for the use of an increasing percentage of the inedible fat production is that of chemical derivatives. Thus the development of industrial chemicals from inedible fats that are useful in industries that require large quantities of chemical raw materials is an area in which research is needed. At the same time, basic and exploratory research on the organic and physical chemistry of fats and fatty acids is needed in order to maintain a continuing supply of fundamental knowledge; to establish new principles and concepts; and to discover new reactions and processes for future development.

Major objectives of the research are to develop:

1. Fundamental knowledge of the composition, structure, and physical and chemical properties of inedible fat and its components.
2. New and improved techniques for separating and characterizing the components and derivatives of fat.
3. New chemical intermediates.
4. New and improved industrial products--particularly for use as biodegradable detergents, plastic foams and coatings and lubricant components and additives.

## Progress - USDA and Cooperative Programs

RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

### A. Chemical Composition, Physical Properties and Structure

1. Computer-based Procedures. To facilitate studies of the solid state behavior of long-chain fatty molecules a series of linked programs was completed for calculating from dielectric data, the parameters of the Cole-Cole equations and the thermodynamic constants of the relaxation-time equation.

As an aid to be used in locating chemically active sites within a fat-based molecule a program has been written which displays molecular orbitals on the curve plotter in a form that gives the visual impression of an electron cloud

of variable density. A pictorial display of an electron cloud distribution reveals structure features of the probability distribution that are not readily apparent in the mathematical functions themselves. This is very helpful in investigating molecular orbitals, since the typical molecular orbital consists of several mathematical terms whose combined effect cannot be easily perceived by merely looking at the equations.

A computer program has been established for determining specific heat of pure glycerides by using the data from differential scanning calorimetry. The program prints out the specific heat, plots the graph of specific heat versus temperature, evaluates confidence limits for the curve, and prints out the best equation of the curve. Specific heats for polymorphic forms of tri-stearin, tripalmitin, trimyristin, and trilaurin have been obtained. The computer program saves time and allows calculation of the equation describing the data to determine the precision. These last two items would not be determined adequately by old-fashioned methods.

A previous EU program for computing spatial locations of the atoms of fat-like chain molecules subject to internal rotation has been revised for the 1130 computer so that 26,000 rotational isomers can be retained on disk storage for further study. Storage allows later computation of molecular bulk and shape, dipole moment, intramolecular interactions, moments of inertia, etc., as affected by rotation through a large number of conformations. Furthermore, since the information on the disk can be accessed in any order, sampling procedures can be investigated as a means for estimating these molecular properties.

Contract studies on spatial interrelations within molecules was completed during the year at the Villanova University, Villanova, Pa. Quantum mechanical procedures were developed and tested for investigating theoretically the most stable conformations of molecules and barriers to internal rotation. Computer programs were developed for molecules of up to 50 atoms, which should be applicable to small triglycerides. The results suggest that the major source of the barrier to rotation is the variation of net charge on each atom as the molecule rotates internally.

2. Separation Techniques. Equations were derived which allow the calculation of the number of tubes required for any desired degree of separation of two solutes such as fatty acid esters in frontal countercurrent distribution (CCD). The CCD equations allow optimization without trial-and-error experimentation.

A combination of thin-layer and gas-liquid chromatography (GLC) was used to obtain fatty acid, sterol and triglyceride composition of natural materials. Investigations were carried out on the determination of lipid distribution in light and dark pork muscles and on separation of isopropenyl esters of fatty acids. Cooperative studies on GLC were carried out with AOCS and ASTM. Consultations were held with the Codex Committee (WHO/FAD).

In research at Technical University, Gdansk, Poland, under a PL-480 grant, the method previously reported for comparing the thermal stability of various



liquid phases for gas liquid chromatography was further clarified and appears to be a practical procedure.

3. Spectroscopy. The research on the molecular structure of saturated and unsaturated fatty acids and their esters by spectroscopy was terminated. The study showed that NMR spectroscopy in conjunction with other physical means constitutes a sensitive technique to obtain detailed structural information on unsaturated fatty acids.

## B. Chemical and Physical Investigation to Improve Products

1. New Chemical Intermediates. The synthesis of isopropenyl stearate was further studied for its potential development on an engineering scale. Iso-propenyl esters continue to show versatility in their reactions, the latest being their conversion to 1,3-diketones. These latter compounds form metal chelates. The unique solvent hexamethylphosphoratriamide permits almost quantitative yields of  $\alpha$ -branched chain acids from reactions on metalated straight-chain fatty acids. Studies on thiocyanogen addition to olefins in various solvents now permit synthesis of either the dithiocyanate or the thiocyanate-isothiocyanate adduct as the major adduct. Development of new peracids and their kinetics of epoxidation are being continued with the aim of finding peracids having specific oxidizing capabilities.

2. Detergents. Direct esterification of mannitol or sorbitol with  $\alpha$ -sulfo-palmitic or stearic acid gave good yields of the 1,6-diester. Acid chloride or alcoholysis methods gave monoesters of hexitols and sucrose. The  $\alpha$ -sulfo esters are biodegradable, have moderate foaming and detergent properties, excellent metal ion stability and good lime soap dispersing properties. Sulfated alkanolamides were prepared by the alcoholysis of methyl laurate, palmitate or stearate with a variety of alkanolamines. Products such as sodium salts of sulfated ethanolamides of the long carbon chain fatty acids were the most stable to hydrolysis and had the best foaming, detergent, and lime soap dispersing properties. Laboratory washing tests with tallow alcohol sulfates showed the usual phosphate builder could be partly or wholly replaced by citrates or nitriloacetates.

Dichlorooctadecanol was desired as an intermediate for the easily soluble biodegradable detergent sodium 9,10-dichlorooctadecyl sulfate. Attempts to obtain it by additive chlorination and catalytic hydrogenolysis were unsuccessful. Use of diborane gave excellent yields in the reduction of chlorinated esters to the dichloro alcohol.

A method was developed to follow the course of aerobic biodegradation of anionic compounds by automated analyses for carbon, methylene blue active substance and sulfate formation. Anaerobic tests on detergent solutions in Schuylkill River water showed tallow-based detergents to be more completely degraded than linear alkylbenzenesulfonates.

Contract studies on the hydrogenolysis of animal fats to alcohols without chain saturation was completed during the year at Swift and Company, Chicago,

Illinois. Optimum conditions considering cost, yield and quality were the hydrogenolysis of "red oil" grade oleic acid to oleyl alcohol in 1 hour at 300°C. and 2500 pounds per square inch pressure, with a catalyst composed of equal parts Al, Cd, Cr, and Zn. Further laboratory and pilot plant research would be necessary before manufacturing feasibility can be established.

In research at the University of Bombay, India, under a PL-480 grant, sulfated monoglycerides were obtained in good yield and purity by sulfation of tallow fatty acid monoglycerides with excess chlorosulfonic acid. The products are not soluble enough for use in liquid detergents. Preliminary experiments show combinations of sulfated tallow monoglycerides with soap have good properties for use in hard water.

3. Lubricants. Synthetic procedures for preparing fatty aziridines were studied. Dichlorourethane gives somewhat lower yields than the iodine-isocyanate method, but presents considerable economies in reagent costs and in the number of processing steps. Addition of N,N-dibromobenzenesulfonamide to olefins followed by reduction and cyclization gives rise to substituted aziridines in fair yields which can probably be improved. A group of soaps of 9,10-epiminooctadecanoic acid has been prepared and tested as grease thickeners. Lithium 9,10-epiminooctadecanoate shows promising properties and is being investigated further. The conversion of aziridines to  $\beta$ -hydroxy alkylamido and acylamido derivatives (hydroxyamides) and to oxazolines, and the interconversion of hydroxyamides and oxazolines has been studied. The goal is the preparation of multiproperty lubricant components, particularly grease thickeners, from fats. Toward the same end the nucleophilic ring opening reactions of N-benzenesulfonyl substituted aziridines is being studied to develop methods for incorporating new functional groups in the aliphatic chain. A series of pyrrolidines from secondary fatty amines was converted to quaternary ammonium salts showing surface active properties. Cyanoethylation of mono- and dihydroxy fatty acids and esters has led to mono- and polyfunctional soaps which were tested as grease thickeners and showed encouraging properties.

The development of a synthetic route (free radical addition reaction) to provide a group of  $\alpha$ -branched fatty acid derivatives was realized. A number of the compounds synthesized show some properties of interest for possible application in lubricants. Hydrolytic stability is particularly improved. However, it is believed that industrial application of this synthetic route will require further development effort, particularly in product purification methods.

4. Polymers. Rigid urethane foams with fire-resistant properties have been prepared from polyols of fatty origin. This was accomplished by incorporating chlorine or bromine in a series of fat-based hydroxy compounds. Substrates including tallow, lard, monoolein and triolein were converted into hypohalogenated derivatives and used in urethane foams. Physical properties of the foams were then determined by standard means. The foams could be made more fire-retardant by use of additives but then displayed lower compressive strength. Other methods for preparation of polyols from animal fats are

currently under investigation.

Terpolymers of acrylonitrile, a lower alkyl acrylate, and n-octadecyl acrylate, have now been found to produce highly flexible, internally plasticized materials having excellent low-temperature properties. In the past, copolymers of acrylonitrile and fatty comonomers yielded only brittle, intractable materials. The quality of resilience can be conferred at high octadecyl acrylate content by the experimental control of side-chain crystallinity.

Side-chain crystallinity studies were focused on selected homopolymer homologs of varied structure. Apparent heats of fusion were determined on the higher vinyl monomers, their homopolymers, and mixtures of homopolymers having 18-carbon side chains. Because the mixed melting temperatures exhibited no depression, and the apparent heats of fusion for the pure homopolymers and their mixtures were the same, it was tentatively concluded that only the alkane portion of the homopolymer enters the crystal lattice.

Studies on the thermolytic cleavage of fatty ozonides carried out under grant at the University of Minnesota, Hormel Institute, Minneapolis, Minn., are essentially completed. The thermal decomposition of triglyceride ozonides in the presence of fatty materials leads to polymeric materials. The reaction may have particular value with animal fats, because these contain considerable amounts of monounsaturated chains which appear to be a more useful source of radicals than more highly unsaturated fats. Furthermore, this is the first instance in which largely saturated fats have been made to polymerize.

5. Stability. Under grant research at Rutgers State University, New Brunswick, N. J., the stability of commercial fatty acids is being investigated. Representative samples of tallow and oleic acid made from tallow were obtained from each of ten fatty acid manufacturers and pooled. Tallow samples were combined as were oleic acid samples to give starting material for study. Minor constituents have been obtained from the composite of oleic acid and have been separated into polar and nonpolar fractions. Components causing color deterioration were concentrated in the polar fraction. Each fraction is being further fractionated in an effort to obtain pure components for identification. A new process for the manufacture of fatty acids appears feasible. This new method produces a product with improved color stability.

In research at Technical University, Gdansk, Poland, under a PL-480 grant, a study of the kinetics of the autoxidation of methyl linolenate showed the reaction to proceed similarly to that of methyl linoleate.

6. Triglyceride Syntheses. Under a grant to the University of Connecticut, Storrs, Conn., the following racemic glycerides of highest purity (98+%), both as to fatty acid composition and positional integrity, were synthesized: glycerol-1-palmitate-3-linoleate, glycerol-1-palmitate-2-linoleate-3-stearate, and glycerol-1-palmitate-2-myristate-3-stearate. The synthesis of linoleate glycerides was extremely difficult because of their great susceptibility to oxidation and their tendency to produce low yields in the purification steps. The use of antioxidants, together with special techniques such as carrying

out entire operations under an atmosphere of nitrogen, helped considerably. The purity of the glycerides at all stages was monitored by TLC, GLC, and pancreatic lipolysis. These high purity glycerides are now available for comprehensive studies on their physical and chemical properties.

C. Technology - Process and Product Development

Research has been initiated to develop and optimize a commercially feasible process for production of enol esters from fatty acids.



## Publications and Patents - USDA and Cooperative Programs

RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

### Chemical Composition, Physical Properties and Structure

Anderson, L. V., Fink, K. H., Herb, S. F., Koester, W. H., Kuiken, K. A., Schmadeke, D. W. and Doughtie, R. T. Jr. 53d annual report of the Smalley Committee 1967-1968. J. Am. Oil Chemists' Soc., 45, (1968), 294A and 299A.

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## HIDES AND LEATHER UTILIZATION

## USDA and Cooperative Program

Problem Area		Scientist Man-Years		
		F.Y. 1969		
No.	Title and Activity	Intra-mural	Extra-mural	Total
410:	New and improved meat, milk and egg products			
	Chemical composition and physical properties	4.9	0	4.9
	Color, texture and other quality factors	2.7	0	2.7
	Technology - process and product development	1.0	0	1.0
	Subtotal	8.6	0	8.6
411:	New and improved products from wool, hides, skins and animal fats			
	Chemical composition physical properties and structure	4.6	0.4	5.0
	Chemical and physical investigations to improve products	8.2	0	8.2
	Microbiology and toxicology	0	0.5	0.5
	Technology - process and product development	6.9	0	6.9
	Subtotal	19.7	0.9	20.6
702:	Protect food supplies from harmful microorganisms and naturally occurring toxins			
	Microbiology and toxicology	0.7	0	0.7
	Subtotal	0.7	0	0.7
	TOTAL	29.0	0.9	29.9

Domestic program is supplemented by PL-480 funds in five countries totaling 458,741 U. S. dollars equivalent (Finland, India, Israel, The Netherlands and United Kingdom).



## Problems and Objectives

Finding outlets for hides has been a continuing problem even since synthetic substitute leather-like materials began to replace leather. The problem has been further compounded by the increase of hide production as a result of the continuing increase in the slaughter of cattle. In order to maintain current markets and provide for new and economical ones for this important product of the cattle industry, which accounts for 6-10% of the live weight of the cattle a strong research program is necessary. Areas in which the current research program seeks to improve the market for hides are in the development of leathers with new and unique properties and in the development of food uses. At the same time a strong basic and exploratory research program is needed in order to maintain a continuing supply of fundamental knowledge regarding the physical and organic chemistry of collagen which is necessary for future developments.

Major objectives of the research are to develop:

1. Fundamental knowledge of composition, structure, and physical and chemical properties of hides and its components.
2. Procedures for attaining high quality raw stock.
3. New and improved techniques for processing hides into leathers.
4. Chemical modifications of hides that result in the development of leathers with unique and desired characteristics.
5. Edible uses for the collagen of cattle hides.

## Progress - USDA and Cooperative Program

### RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

#### A. Chemical Composition, Physical Properties and Structure

1. Structure of Collagen. Techniques were developed to prepare films of undenatured collagen which are suitable for studies with infrared spectroscopy. Infrared spectra of native collagen have shown that a considerable amount of water is tightly built into the native structure, i.e., the water has lost its usual characteristics. These data also suggest that there are two distinct types of carbonyl groups in the native backbone of collagen.

NMR studies of many amino acids have shown that the temperature dependence of anion rotamer energies is characteristic for all aromatic amino acids. The variations with concentration appear to be related to the polarity of the aromatic group and may reflect changes in hydrophobic interactions, a possible important structural aspect of collagen.

Under grant research at Northwestern University, Evanston, Ill., it was found that acid-soluble bovine corium collagen forms upon denaturation a much more complex mixture of gelatins than rat skin collagen. Even after chromatography on a carbomethoxycellulose column and disc electrophoresis the fractions are not monodisperse, and further fractionation after pepsin treatment shows that polymeric material is present in some of the chromatographed fractions. A technique for solubilizing corium collagen based on reducing this material to a finely divided state prior to placing it in contact with buffer has been developed and gives yields up to 20%, although a large portion of this is polymeric. A new hypothesis was developed to explain the three-dimensional ordering of monomeric units within the collagen fibril.

Under a PL-480 grant to the Central Leather Research Institute, Madras, India, the study of shrinkage characteristics of skin and collagen was continued. Age-dependent changes in shrinkage behavior were found to be due not to accumulation of trace amounts of metals but probably due to the accumulation of metabolic aldehyde intermediates. Contrary to reports in the literature, the amino acid hydroxyproline is not liberated when collagen from rat-tail tendon is denatured by heating in water. Thermodynamic parameters (heat and entropy of fusion) increase with introduction of chemical cross-linking.

At the University of Turku, Turku, Finland, under a PL-480 grant, comparison of collagens from various species revealed that heat stability has been an evolutionary prerequisite for the development of homothermic animals. As collagen matures, it becomes more insoluble. Collagenous tissue contains a gradation of cross-linking and can be fractionated with dilute acid over a thermal gradient. The bulk appears in fractions at 35-40°C. and at 70-80°C. Insoluble collagens may also contain within their matrix a series of glycoproteins, which can be isolated after degradation of the collagen with collagenase.

2. Structure of Hides. An experimental lot of Hereford steerhides is being characterized with respect to incidence of the vertical fiber defect, which seriously impairs strength. Correlation of results with genetic background will afford suggestive evidence as to whether the defect is heritable.

3. Electrolyte Determination in Hides. Application of specific ion electrodes to the determination of hide composition and rates of penetration of solutions into various areas and strata of hides is continuing. The silver sulfide electrode is affected by pH changes and means for correcting the electrode potential to obtain true concentration values are being studied. Electrolysis at constant current may provide a means for determining total free and combined electrolytes in a hide or leather. The use of surface glass electrodes and specific ion electrodes can determine in a nondestructive manner some of the variables which cause non-uniformity in animal hides.

## B. Chemical and Physical Investigations to Improve Products

1. Modification of Collagen. The reaction of formaldehyde and malonic acid with proteins has been extended to include wool keratin, casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin. Chemically, the results are similar with the formation of most of the same products as are formed with collagen. The latter two proteins have between 3 and 4 times more lysine than collagen which makes the derivatives easier to detect. Progress has been made in isolating the more abundant of these by a combination of gel filtration and ion exchange chromatography. It is probable that intermolecular cross-linking is taking place in most of these proteins. With collagen, the increase in shrinkage temperature is indicative of this, while precipitation takes place with soluble proteins.

At the Central Leather Research Institute, Madras, India, under a PL-480 grant a method was developed for differentiating between grafting of monomer with collagen and homopolymerization of monomer, involving detection of amino acid end groups in the graft isolated after acid or enzymatic hydrolysis of the graft polymers. Low concentrations of ceric ion and of monomer favored grafting. An indication of the mechanism of grafting was obtained by prior blocking of selected reactive sites. The hydroxyamino acids and the peptide backbone of collagen may be involved in the mechanism of grafting. Alginic acid was coupled with the lysine residues in gelatin, giving products which formed excellent, pliable, transparent, continuous films having excellent adhesive properties.

Under another PL-480 grant at the Central Leather Research Institute, Madras, India, studies have been made of the isotopic exchange of the mono- and bisulphato chromium complexes. Variation of the chrome concentration and ionic sulfate did not affect the rate of exchange but the exchange rate was found to increase with increase of pH. Infrared spectra could not distinguish between the two di-nuclear chromium complexes studied. The polynuclear complexes from 33% chrome liquor produced four bands on a carboxymethyl cellulose column and fractionation and characterization are in process. Diffusion of chromium through collagen membranes is dependent upon temperature and thickness and independent of the chrome content.

2. Properties of Leather and Leather Containing Products. Standard chrome-tanned leather and glutaraldehyde retanned leather have been dyed with reactive dyes and the leather drycleaned with each of the three common drycleaning solvents. The colors tested were fast in all three.

Shearlings dyed with this reactive dye can be made fast to washing but in all experiments so far, the wool shade is much lighter than the leather.

A commercial long-chain fatty amino acid has been found to be an effective softening agent when used as an after-rinse in the washing of shearlings. Retannage with a minimum of 2% glutaraldehyde appears necessary to impart perspiration resistance to chrome leather. Resistance appears to be improved when using larger amounts of glutaraldehyde. The finishing of the



leather (fat liquoring, etc.) also appears to be important in affecting resistance.

Hydration properties of various leathers were also studied to demonstrate the effect of certain tanning factors, i.e., glutaraldehyde retannage, alkenyl succinic acid lubrication, and water repellency. Treatment with water repellent agents did not lower permeability or absorption of water vapor but markedly lowered absorption of liquid water. Retannage with glutaraldehyde or lubrication with alkenyl succinic acid showed little effect on any of these three properties of the leather.

Dehydration of hides is attractive from the standpoint of preservation and transportation of this raw material. Dilute aqueous solution of alkylene glycols and derivatives offer promise of effecting this objective. Further study is needed to determine the economic feasibility of such a process, and to overcome the poor break and pipey grain of the leather made from hides dehydrated in this process.

Under a PL-480 grant at the Central Leather Research Institute, Madras, India, the temperature and humidity within shoes while being worn have been measured. With 30°C. room temperature, the temperature within the shoe tended to reach body temperature within two hours and the relative humidity between foot and shoe increased from 70% to over 90% after eight hours. Use of a moisture barrier film caused the humidity to reach 100% and the temperature went above body temperature. The latter conditions were uncomfortable to the wearers while the former were not considered uncomfortable. Studies showed that extracts from vegetable-tanned sole leathers reduced the growth of bacteria in shoes but that perspiration tended to decrease this effect.

#### C. Microbiology and Toxicology

Under contract research at the Southern Research Institute a microbiological study of shearling laundering and disinfecting procedures is being studied. These studies involve the potentially infectious agents associated with shearling bedpads (woolskins). The efficiency of various type laundering and disinfecting methods currently used in the cleaning of shearling bedpads, using selected pathogenic viruses (vaccinia and poliomyelitis) and bacteria (Staphylococcus aureus and Pseudomonas aeruginosa) are being investigated. Laundering in anionic and nonionic detergents in combination with quaternary ammonium, phenolic or glutaraldehyde disinfectants were effective in reducing vaccinia virus on both wool and leather.

#### D. Technology - Process and Product Development

1. Leather Manufacturing Operations. Bench-scale laboratory equipment has been set up which simulates the presently used methods of bating, pickling, and tanning of hides during the manufacture of side leather. Experiments have just been initiated to study the effect of mechanical agitation on the rate of diffusion of chemicals into the hides during bating, pickling, and chrome tanning; to quantitatively measure the chemical changes which occur;



to determine the effect of the chemical changes on the physical characteristics of the hide materials; and to describe the rates of diffusion and the reaction rates by means of mathematical equations. The information obtained will be used to develop improved processing techniques and/or equipment for manufacturing side leather.

2. Elimination of Skin Defects. Completion of the cooperative test on living sheep established conclusively that keds (sheep ticks) are the cause of the sheepskin defect known as "cockle." A second test is underway to estimate the potential economic value of ked eradication to the tanning, wool-pulling and sheep-raising industries.

3. Tanning Investigations. Work was completed on the use of glutaraldehyde in producing products of superior durability. The perspiration resistance of work glove leather was improved and woolskins proved more durable in hospital tests when tanning reagents included glutaraldehyde.

Under a PL-480 research grant at the Central Leather Research Institute, Madras, India, a four-pit tanning system was devised for the production of sole leather. Tannage in the pits took four days, followed by drum tannage for 18 hours. The properties of the leather compared favorably with I.S.I. specifications. Limed hide was pretreated with sodium nitrite and formic acid for 24 hours and then put into wattle liquor. Penetration was over in 3 days. The use of sodium sulfite in the pretreatment instead of sodium nitrite was not as effective, however penetration was over in six days as against 9-10 days for the control.

Tanning shearlings by swabbing a paste formulation of glutaraldehyde on the flesh side produces satisfactory leather. Tanning is rapid and the wool remains white except on the edges. Formulations are being studied to reduce the time and care necessary in this manual procedure. The same procedure is being applied to rabbit skins but the yellow color of the skin shows through the fur.

## RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

### A. Chemical Composition and Physical Properties

1. Characterization of Solubilized Collagen. The apparatus used for electric birefringence measurements of solubilized collagen was redesigned and expanded so as to give a higher power output. The measurements showed that collagen's permanent and/or induced dipole moments are functions of the following: pulse amplitude and pulse width, ionic strength, pH, dielectric constant and viscosity of the solvent, and concentration of collagen and age of the preparation. A study of the relationships between these parameters has been begun. Decay of electric birefringence of solubilized collagen and its related rotatory diffusion constant are functions of initial birefringence and collagen concentration. Disc electrophoresis was carried out at intervals during collagen solubilization runs and showed the unexpected result that there is no variation in the composition of the collagen

solubilized at different stages in the process. This new approach to the study of collagen promises to yield extensive information on its structure and physical properties, and to provide a much-needed criterion of the monodispersity of solubilized collagen preparations.

## B. Color, Texture and Other Quality Factors

1. Fibrous Dispersions of Collagen. Conditions have been established under which delimed cattlehide flesh splits can be ground and shredded to retain the fibrous character. Hide collagen has been ground to produce a bacteriologically and organoleptically satisfactory food protein at 25-35% solids. In this form it is readily mixed with supplemental foods or dispersed at about pH 4, 20% solids.

Collagen dispersions have been cross-linked with glyceraldehyde to produce a heat-resistant film and "tanned" collagen has been sent to WU for rat-feeding tests to determine digestibility, nutritional value and toxicity. Glyceraldehyde is being tested alone for toxicity.

## C. Microbiology and Toxicology (no current research)

## D. Technology - Process and Product Development

1. Process for Comminuting Cattlehide. A batch process for comminuting cattlehide has been developed. It consists of 5 steps: (1) washing the hides, removing some fat, and adjusting the pH; (2) precutting into strips in a strip cutter; (3) cutting the strips into 1-inch squares by means of a rotary knife cutter; (4) further comminution in a grinding mill to a maximum particle size of .06 inch; (5) pulping by application of a shearing force inside a disc mill. The product from step (4) consists of granules and fibers which readily agglomerate. The pulp obtained in step (5) contains fiber bundles and fibers whose length depends on the size of the particles fed to the disc mill; but there are no hard lumps present in this product. It has been found necessary to cool the equipment used in the last two steps to prevent denaturation of collagen. Products with solids contents ranging between 20 and 30% have been made. Differential thermal analysis has proven useful for detecting denaturation of collagen, and the results obtained by differential scanning calorimetry indicate that this method of analysis will permit quantitative measurement of the proportion of the total amount of collagen which has been denatured during processing. A screen test has also been devised to measure the size distribution of particles in the product from step (4). It involves tanning prior to screening to minimize changes in particle size induced during testing. Viscosity measurements were made using a Brookfield viscosimeter, however, the results are of questionable accuracy.

2. Food Applications. Mixtures of collagen with meat have proven palatable. Industry has been supplied with collagen for testing in food applications and in pet foods.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS  
AND NATURALLY OCCURRING TOXINS

A. Chemical Composition and Physical Properties (no current research)

B. Color, Texture and Other Quality Factors (no current research)

C. Microbiology and Toxicology

A micro-method for identifying single colonies of members of the Enterobacteriaceae was developed and is being evaluated. The method is approximately seven times as rapid as the conventional macro-method and can be used to identify single colonies with an incubation time of about one day. Conventional methods require two days for Salmonella confirmation.

A comparison was made on the use of a single large sample of meat and bone meal versus ten smaller samples. Indications were that the two methods were not significantly different.

D. Technology - Process and Product Development (no current research)

## Publications - USDA and Cooperative Programs

### RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

#### Chemical Composition, Physical Properties, and Structure

- Cavanaugh, J. R. Conformational stability of phenylalanine in aqueous solution by nuclear magnetic resonance. J. Am. Chem. Soc., 90, (1968), 4533-4536.
- Everett, A. L., Willard, H. J., and Windus, W. Microscopic study of leather defects. II. Inherent vertical fiber structure in side leather. J. Am. Leather Chemists' Assoc., 62, (1967), 25-44.
- Heikkinen, E., Valavaara, M., Penttinen, R., and Kulonen, E. The preparation of various components of collagen. Ann. Med. exp. Fenn., 45, (1967), 259-263.
- Lampiaho, K., Niinikoski, J., Kari, A., and Kulonen, E. Fractionation of pepsin-digested, denatured collagen. Biochim. Biophys. Acta, 160, (1968), 77-89.
- Lampiaho, K., and Kulonen, E. Metabolic phases during the development of granulation tissue. Biochem. J., 105, (1967), 333-341.
- Subbaraman, L. R., and Ramaswamy, D. Self-diffusion of cationic chrome complexes through cationic exchangers. J. Am. Leather Chemists' Assoc., 63, (1968), 246-257.
- Pikkarainen, J. The molecular structures of vertebrate skin collagens. Acta Physiol. Scand. Suppl., 309, (1968), 1-72.
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- Witnauer, L. P., and Palm, W. E. Influence of cyclic conditioning on the hydrothermal stability of leather. J. Am. Leather Chemists' Assoc., 63 (1968), 333-345.

#### Chemical and Physical Investigations to Improve Products

- Happich, W. F., Windus, W., and Naghski, J. The moisture sorption properties of glutaraldehyde-chrome-tanned shearlings. J. Am. Leather Chemists' Assoc., 62, (1967), 718-732.



Happich, M. L., Naghski, J., and Windus, W. The application of glutaraldehyde to tanning belly work glove leather. J. Am. Leather Chemists' Assoc., 63, (1968), 74-89.

Luvisi, F. P., and Filachione, E. M. The effect of glutaraldehyde on vegetable tanning. J. Am. Leather Chemists' Assoc., 63, (1968), 584-600.

Windus, W., and Showell, J. S. An interpretation of the mechanism of unhairing as a nucleophilic displacement. J. Am. Leather Chemists' Assoc., 63, (1968), 258-274.

#### Technology - Process and Product Development

Everett, A. L., Naghski, J., Farrell, R. K., and Winward, L. D. Microscopic study of leather defects. III. Structural changes induced by freeze-branding. J. Am. Leather Chemists' Assoc., 63, (1968), 614-630.

Everett, A. L., Willard, H. J., Bitcover, E. H., and Naghski, J. Cockle defect of sheepskin: Its nature, monthly incidence and severity in domestic woolskins. J. Am. Leather Chemists' Assoc., 64, (1969), 150-163.

Muthiah, P. L., Ramanathan, N., and Nayudamma, Y. Physical properties of shoe upper materials and their effect on footwear comfort. J. Indian Leather Tech. Assoc., 16, (1968), 78-85.

Thampuran, K. R. V., Balakrishnan, M., and Ghosh, D. Studies on the rapid tannage of heavy leather. Leather Sci., 15, (1968), 143-149.

### RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

#### Chemical Composition and Physical Properties

Kahn, L. D., and Witnauer, L. P. Electric birefringence of collagen. J. Am. Leather Chemists' Assoc., 64, (1969), 12-18.

#### Color, Texture and Other Quality Factors (none to report)

#### Microbiology and Toxicology (none to report)

#### Technology - Process and Product Development (none to report)

### RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

#### Chemical Composition and Physical Properties (none to report)

#### Color, Texture and Other Quality Factors (none to report)

#### Microbiology and Toxicology (none to report)

#### Technology - Process and Product Development (none to report)

## POTATO UTILIZATION

## USDA and Cooperative Program

Problem Area		: Scientist Man-Years		
		: F.Y. 1969		
No. :	Title and Activity	Intra- :	Extra- :	Total
:		mural	mural	
403 :	New and improved fruit and vegetable	:	:	:
:	products	:	:	:
:	Chemical composition and physical	:	:	:
:	properties	4.3	:	:
:	Color, texture and other quality	:	:	:
:	factors	4.2	:	:
:	Technology - process and product	:	:	:
:	development	2.1	:	:
:	Subtotal	10.6	:	:
:		:	:	:
901 :	Alleviate soil, water and air pollution	:	:	:
:	Technology - process and product	:	:	:
:	development	5.5	:	:
:	Subtotal	5.5	:	:
:	TOTAL	16.1	:	:

Domestic program is supplemented by PL-480 funds in one country totaling 36,854 U.S. dollars equivalent (Poland).

## Problems and Objectives

The welfare of the potato industry depends on continuing the present trend to use increasing amounts of the potato crop for processing into products such as chips, French fries and dehydrated potatoes. Processing of potatoes, however, poses several problems, the most important being disposal of potato wastes. The potato starch plants, which provide growers and processors with an outlet for cull potatoes, as well as other processors may be forced out of business by antipollution laws. Recovery of useful materials from potato wastes would help alleviate the potato waste problem and provide additional return to the processor. Another area of concern relates to predicting and controlling processing characteristics of potatoes. Potatoes placed in cold storage to extend the processing season require a lengthy reconditioning treatment which is not always successful. Research is also needed to provide new and improved processed potato products.

Major objectives of present research are:

1. Developing technology for recovery of useful materials, including protein and amino acids, from potato waste.
2. Developing technology for evaluating potatoes for processing, controlling browning during processing, and predicting quality at time of consumer use.
3. Perfecting the explosion-puffing process to provide new and improved dehydrated potato products.

## Progress - USDA and Cooperative Programs

### RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

#### A. Chemical Composition and Physical Properties

1. Enzymes and Interconversion of Sugars. Sucrose synthetase has been characterized and the level in developing tubers followed for a single growing season. It was relatively high during development of tubers and dropped sharply at tuber maturity. Sucrose synthetase, or one of its ancillary components, or a hormone may be the long sought for index of maturity. ("Immature" tubers do not recondition as well as "mature").

The carbohydrate transformation pathway in cold and warm tubers is essentially worked out. Work on methods of control is being initiated, involving the study of hormones, growth regulators, index of maturity, chemicals applied during growth, modification of fertilizers, chemicals applied during storage, new storage techniques, breeding, etc.

Sucrose phosphate synthetase is being characterized. Such studies are handicapped by unavailability of sucrose-6-phosphate. Sucrose phosphorylase, an alternate enzyme to sucrose synthetase in stored tubers is being investigated. With the aid of radioactive sugars, knowledge of some of the pathways of interconversion of sugars has been confirmed and expanded.

2. Potato Proteins. Optimum concentrations for the resolving and stacking gels and conditions for preventing band curvature in disc electrophoresis gels were determined. Freshly prepared potato protein samples produce superior electrophoretic patterns as indicated by 22-24 bands per sample as compared to 15 produced by somewhat aged samples. A staining procedure for locating glycoproteins on the gels was greatly improved by making several significant changes in published methods.

A cooperative potato breeding study to develop a new potato variety of higher protein level was initiated. In the first season analysis of available crosses indicated wide differences in amounts and ratios of soluble (amino acid) and insoluble (protein) nitrogen. Next, to design efficient selection procedures for tuber protein increase, seedlings and parents from ten biparental matings in quintuplicate (300 samples) were analyzed for total nitrogen and total solids. Disc electrophoretic patterns from representative parents and sibs were obtained and are under study.

## B. Color, Texture and Other Quality Factors

1. Texture of Raw Potatoes and French Fries. Cracking of raw potato pieces in shear tests, a sensitive texture measuring method for French fries, makes its use impractical for predicting processed quality from raw material characteristics. The use of the durometer, which measures penetration of a pointed plunger, was studied. Shear and durometer tests were applied to tissues from various locations within the potato tuber. Shear force varied directly and the durometer reading inversely with moisture. Correlation of data from these two tests indicated that the durometer may be a cheaper, more compact instrument for texture prediction than the shear press.

Modified starches have been used to improve French fried potato texture. Samples of two starches under development elsewhere for this purpose were assayed for texture improvement by the shear method. It was concluded that slight increases in textural quality could be detected with one sample and no difference with the other but none of these differences were detectable by a taste panel.

2. Simulated Chip Frying. Investigation continues on the reaction between amino acids and sugars. The relative rates of reaction have been studied in simulated chip-frying. Frying paper discs does not produce as complicated a mixture as results from the same reaction in solvents. The reaction does not follow the first order rate equation and attempts are being made to calculate equations to fit the browning rate curves.



### C. Technology - Process and Product Development

It has been found that the use of a non-condensable gas in conjunction with superheated steam for explosion-puffing will reduce the formation of off-flavors below the taste threshold. Inert gases such as carbon dioxide and nitrogen have proved satisfactory. Air reduces the formation of the off-flavor aldehydes but produces a rancid flavor in the dice. Heretofore, a 3/8" x 3/8" x 3/8" potato piece was the largest size considered to be commercially feasible. By application of the explosion-puffing technique, a much larger size (3/4" x 3/4" x 1/2") piece was successfully produced. This is considered a major break-through in potato dehydration. The large piece will rehydrate in 5 to 6 minutes. The explosion-puffing process has been successfully applied to the eastern Katahdin variety from Maine, Virginia and New York.

## RPA 901 - ALLEVIATE SOIL, WATER, AND AIR POLLUTION

### A. Chemical Composition and Physical Properties (no current research)

### B. Color, Texture and Other Quality Factors (no current research)

### C. Technology - Process and Product Development

A comparison of the flux rates for dilute potato starch waste water of coarse, intermediate, and fine porosity membranes for the reverse osmosis equipment was carried out. The data indicated the intermediate porosity to be the one of choice. It is easily feasible to concentrate the soluble solids in potato waste solution with the upper concentration limit being 6.5% solids because of precipitation of protein. Concentration to 3-4% appears most practical.

The ion exchange study has been divided into two essentially separate steps: the recovery of inorganic cations and the recovery of the amino compounds. This is possible because potassium (from the waste) replaces the amino acids as loading of the column progresses, and the amino acids are displaced from the first column and held by a second column. Elution of each column produces an almost pure inorganic fraction and an amino acid fraction. A fertilizer mixture can be produced from the tailings of the amino acid column and the inorganic cations. The potato waste effluents from the cation and amino acid columns can be further purified by use of a resin type gel. Fifty percent of the remaining solids, 99% of the phosphates, 75 to 90% of the residual ash and 30 to 45% of the remaining chemical oxygen demand can be removed.

Equipment for producing protein water needed for pilot plant studies has been assembled and an experimental unit for studying the heat coagulation of proteins has been developed. Studies have shown that up to 99% of the proteins can be settled out by pH control, thereby reducing the volume to be further processed to 20% of the original.

## Publications - USDA and Cooperative Programs

## RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

Chemical Composition and Physical Properties

Pressey, R. Invertase inhibitors from red beet, sugar beet, and sweet potato roots. *Plant Physiol.*, 43, (1968), 1430-1434.

Pressey, R. Inhibition of invertases by pyridoxal and its analogues. *Biochim. Biophys. Acta*, 159, (1968), 414-416.

Color, Texture and Other Quality Factors

Ross, L. R., and Porter, W. L. Interpretation of multiple-peak shear force curves obtained with French fried potatoes. *Amer. Potato J.*, 45, (1968), 461-471.

Shaw, R., and Lukes, A. C. Reducing the oil content of potato chips by controlling their temperature after frying. U. S. Agricultural Research Service, (1968), ARS-73-58.

General

Ogg, C. L. Report on microchemical methods. *J. Assoc. Offic. Anal. Chemists*, 52, (1969), 248-249.

Ogg, C. L., and Scroggins, L. H. Collaborative study of molecular weight determination applicable to samples with molecular weights less than 500. *J. Assoc. Offic. Anal. Chemists*, 51, (1968), 1231-1236.

Scroggins, L. H. Spectrophotometric microchemical phosphorus determination. A quantitative oxygen flask procedure applicable to problem organophosphorus compounds. *Microchem. J.*, 13, (1968), 385-391.

Scroggins, L. H., and Ogg, C. L. Isothermal molecular weight determination; apparatus, procedure, and study of effect of solvent and standard on accuracy and precision. *J. Assoc. Offic. Anal. Chemists*, 51, (1968), 992-999

## VEGETABLE UTILIZATION

USDA and Cooperative Program

Problem Area		: Scientist Man-Years		
		: F.Y. 1969		
No.	Title and Activity	Intra- mural	Extra- mural	Total
403	New and improved fruit and vegetable products			
	Technology - process and product development	2.1		
	TOTAL:	2.1		

### Problems and Objectives

Economic and social factors continue to accelerate the trend towards processing greater amounts of the vegetable production. Economically, utilization as processed, rather than fresh, vegetables provides a constant source of supply with less price fluctuation. Modern military feeding needs products with high bulk density which do not require refrigeration and are rapidly hydratable. Consumer preference is shifting to "convenience" foods. Thus, emphasis in research is on improvement in processing technology, particularly for dehydrated products.

The major objective of this research is to perfect the explosion-puffing process to provide dehydrated vegetables which rehydrate rapidly, cook in the same or less time than their fresh counterpart, and have good initial flavor and storage stability at room temperature.

### Progress - USDA and Cooperative Programs

#### RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

##### A. Technology - Process and Product Development

Vegetable research during this period was confined to two root crops: sweet potatoes and rutabagas. Processing steps and conditions were determined for processing before, during and after puffing.

Puerto Rican yams were lye-peeled with reasonably low losses and little heat penetration. The enzyme systems were inactivated by steam-blanching. Puffing conditions to give a product with fast rehydration, good color, texture and flavor were determined.

When rutabagas were processed it was found that only slight changes from conditions used for processing yams were needed.



## Patents - USDA and Cooperative Programs

## RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

Technology - Process and Product Development

Eskew, R. K., and Cording, J., Jr. Process for manufacturing rapidly rehydratable dehydrated fruits and vegetables of high density. U. S. Patent 3,408,209, October 29, 1968.

Heiland, Wolfgang K. Self-cleaning venting orifice. U. S. Patent 3,430,643, March 4, 1969.

## DECIDUOUS FRUIT AND TREE NUT UTILIZATION

USDA and Cooperative Program

Problem Area		:Scientist Man-Years		
		: F.Y. 1969		
No.:	Title and Activity	: Intra-	: Extra-	:Total
		: mural	: mural	
403:	New and improved fruit and vegetable products	:	:	:
:	Chemical composition and physical	:	:	:
:	properties	: 0	: 0	: 0
:	Flavor	: 1.2	: 0	: 1.2
:	Color, texture and other quality factors	: 1.7	: 0.6	: 2.3
:	Technology - process and product	:	:	:
:	development	: 3.4	: 0	: 3.4
:	TOTAL	: 6.3	: 0.6	: 6.9

## Problems and Objectives

Continued improvement in the quality of processed fruits is essential if fruit is to hold its own as an element in the national diet. Mechanical harvesting and other changes in the growing and harvesting of fruits create problems which must be met by improved processing methods. The preservation of fruit juices with good flavor, color, and other properties is essential in maintaining good markets for these products. The development of new fruit varieties more suitable for various types of processing is an important factor in preserving markets for the fruit processing industry and protecting fruit growers against variations in price due to irregular yields from year to year.

Objectives of the research are:

1. To develop high quality dehydrated fruit products which may be reconstituted quickly, and which may be consumed as snacks.
2. To develop an instant apple sauce of good quality from explosive-puffed dehydrated apples.
3. To develop improved methods for the processing of mechanically harvested fruits and for maintaining the processing quality of fruits harvested by this method.
4. To evaluate the processing characteristics of new fruit varieties.

## Progress - USDA and Cooperative Programs

### RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

#### A. Chemical Composition and Physical Properties

In grant research at Temple University ripe Montmorency cherries, both bruised and unbruised, were analyzed for total alcohol insoluble solids and for their content of pectin, lignin, and cellulose. It was found that bruising and aging (24 hours) caused a 10 to 20% increase in total alcohol insoluble solids in these red tart cherries. Pectin content decreased but lignin and cellulose increased. The metabolic pathways for the synthesis of such high molecular weight carbohydrate material was studied by means of C<sup>14</sup>-labeled metabolites. Injection of C<sup>14</sup>-labeled metabolites into bruised cherries showed that acetate was metabolized faster than citrate, and citrate faster than glucose.

#### B. Flavor

The aromas of fresh Montmorency cherry juice and a 150-fold commercial cherry essence were evaluated by a small taste panel and by GLC. The two aromas were

found to be quite dissimilar. When fresh juice was heated it developed a typical cooked "cherry pie" aroma. The essence prepared from this juice possessed a "cooked cherry" note plus a "plum-like" or "lemon" character. On GLC examination, a benzaldehyde peak was prominent in both fresh juice and essence from the cooked juice, but the other peaks were quite dissimilar.

Organoleptic evaluation of the various GLC peaks of cherry essence revealed that the low-boiling compounds contributed a "fresh, fruity" character; the intermediate compounds were more representative of the essence aroma, while the high-boiling compounds were more "lemon-like." All three fractions were necessary to the complete aroma.

#### C. Color, Texture, and Other Quality Factors

A total of 48 new pear varieties, resistant to fire blight, were evaluated for canning suitability in contract research conducted at Rutgers University. The fruit was harvested, stored, ripened, and processed by canning. At least seven of the new varieties were equal to or better than Bartlett pears in texture and flavor.

Studies on improved methods of handling machine-harvested cherries show that maximum yield and optimum quality are achieved when the fruit is chilled promptly in the field tanks and when rehandling is avoided. Procedures were developed for estimating the weight of cherries by measuring the volume in the field tanks. One large processor last season purchased over 10 million pounds of cherries by volume rather than by weight. In 1968, approximately 70% of the U.S. crop of red tart cherries was harvested by machine, saving \$7 million in labor costs. In addition, an estimated 15% of the crop, valued at \$4 million and too sparse to hand-pick, was salvaged by machine-harvesting.

#### D. Technology - Process and Product Development

Ultraviolet (UV) irradiation of apple cider to increase shelf life has been applied on a commercial scale, one fruit grower processing 40,000 gallons. The cider retains its original flavor, and the microbial count is reduced to a very low level without the use of heat or preservatives. Storage life is further markedly extended by adding a trace of potassium sorbate (amounts below the taste threshold) to maintain the low microbial count. Presence of the sorbate in the cider reduces the effectiveness of the UV treatment, so best results are obtained by the combination of UV irradiation followed by adding sorbate.

There is considerable variation in effectiveness of the lye-peeling process with the variety of apple and conditions must be varied accordingly. Also, the time required for dewaxing and lye-peeling a particular variety increased with the length of time held in cold storage.

Rome Beauty variety apples, obtained from controlled atmosphere storage, were successfully explosive-puffed. Pies and apple snacks made from this variety were of excellent quality.



Cooperative work was continued with Musselman Fruit Products Division, Pet Inc., to evaluate dehydrated applesauce and slices made by the explosive-puffing process. Market tests were made by commercial firms using the dried sauce as a recipe ingredient in baking items and candy. Cafeteria feeding tests of reconstituted sauce and slices were also carried out. From the results of these tests it was concluded that the quality of the dehydrated products in their present form was acceptable.

The preparation of a rapidly rehydratable apple half segment was accomplished by development of an alternative procedure to explosive-puffing. The procedure consists of freezing the pieces followed by drying in hot air to about 23% moisture, then drying in hot air or vacuum to low moisture. Tests on York Imperial variety apples showed a marked improvement in rehydration over conventionally dried products.

Bartlett variety pears were used to prepare dehydrated, explosive-puffed, pear dice. The product obtained was well puffed, rehydrated rapidly and retained good color and flavor upon rehydration. When compared to a commercially prepared dice, the explosive-puffed product was more easily rehydrated and of more acceptable color and flavor.

## Publications - USDA and Cooperative Programs

## RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

Color, Texture and Other Quality Factors

Diener, R. G., Whittenberger, R. T., and Levin, J. H. Preharvest spray cooling for tart cherries. Mich. Agr. Expt. Sta. Res. Report, 69, (1968).

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Hills, C. H., and Whittenberger, R. T. Mechanization comes to the harvesting of red tart cherries. Candy Ind. and Confec. J., 132, No. 5, (1969), 13-14.

Tennes, B. R., Levin, J. H., and Whittenberger, R. T. New developments in mechanical harvesting of apples in Michigan. New York State Hort. Soc Proc., 114, (1969), 115-121.

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Whittenberger, R. T., Levin, J. H., and Gaston, H. P. Maintaining quality by brining sweet cherries after harvest. Mich. Agr. Expt. Sta. Res. Report, 73, (1968).

Technology - Process and Product Development

Harrington, W. O., and Hills, C. H. Reduction of the microbial population of apple cider by ultraviolet irradiation. Food Technol., 22, (1968), 1451-1454.

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Harrington, W. O., and Hills, C. H. Speeding apple peeling. Canner/Packer, 137, No. 12, (1968), 25.

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## TOBACCO UTILIZATION

## USDA and Cooperative Program

Problem Area		Scientist Man-Years		
		F.Y. 1969		
No.	Title and Activity	Intra-mural	Extra-mural	Total
709	Reduce health hazards in use of nonfood products			
	Chemical composition, physical properties and structure	5.9	5.5	11.4
	Chemical and physical investigations to improve products	4.9	14.7	19.6
	Microbiology and toxicology	3.7	0	3.7
	Technology - process and product development	5.0	2.5	7.5
	Subtotal:	19.5	22.7	42.2
407	New and improved feed, textile and industrial products from field crops			
	Chemical composition, physical properties and structure	0.3	0	0.3
	Subtotal:	0.3	0	0.3
	TOTAL	19.8	22.7	42.5

## Problems and Objectives

Tobacco is grown on about a million acres, and in seven states typically provides more farm cash receipts than any other field crop. The most serious problem affecting the tobacco industry is the concern regarding effect on health.

Major shortcomings in developing a safer cigarette are the inadequacies of biological testing procedures and the failure to know which substances in tobacco and smoke should be removed. There is no practical procedure for determining which substances in tobacco and tobacco smoke are injurious to public health. Based on limited knowledge, some currently marketed cigarettes with charcoal and other filters apparently show selective reductions in certain properties in laboratory animals but cannot be considered a final solution. In addition, such cigarettes are deficient in flavor and aroma and meet with some consumer resistance. This problem requires a concerted effort to learn specifically what must be removed from cigarette smoke and how this can be done effectively without loss of desirable organoleptic properties.

Objectives of research are to:

1. Develop methods for determining which substances in tobacco and tobacco smoke are undesirable from standpoint of public safety, and
2. Develop technology for elimination of substances determined to be undesirable.

## Progress - USDA and Cooperative Program

### RPA 709 - REDUCTION OF HEALTH HAZARDS INVOLVED IN USE OF NONFOOD FARM PRODUCTS

#### A. Chemical Composition, Physical Properties and Structure

1. Composition of Tobacco Smoke. Cigarette smoke condensate was fractionated by the recently developed method to concentrate polynuclear aromatic hydrocarbons (PAH). The PAH-enriched fraction contains at least 480 components, comprising PAH, aromatic secondary amines and insecticides and degradation products thereof. Except for isopropyl-9,9-dimethylacridan and a probable chlorinated hydrocarbon all of the isolated compounds were previously known constituents and probably play no role in tumorigenicity other than that previously suspected. Concurrent bioassay results on the fractions obtained in this isolation procedure showed that a highly polar group of neutral substances of probable high molecular weight may be of greater biological importance than the PAH-containing fraction.



Volatile fatty acids were found in the weakly acidic condensate pigment previously shown to contain quinic acid, iron, amino acids, alkaloids and a silicone.

At the University of Kentucky Research Foundation, Lexington, several composition studies are conducted under contract.

Retention times were obtained for several 3- and 4-ring polynuclear aromatic hydrocarbons which were separated on columns containing graphitized carbon black (gas-solid chromatography). This adsorbent was found to be uniquely suited for separating a mixture of anthracene and phenanthrene on a short column. Certain nitrogen heterocyclic compounds also were separated using paper, thin-layer, and column chromatographic techniques to evaluate various systems for this purpose.

Work also was initiated on the development of an analytical procedure for determining certain heterocyclic compounds in tobacco smoke. Carbazole and dibenz(c,g)carbazole plus several other compounds were isolated.

Preliminary results on the application of gas chromatography, column chromatography, and thin-layer chromatography for the separation of the higher-boiling heterocyclic nitrogen compounds in cigarette smoke were reported. Evidence for the presence of at least 14 compounds was obtained.

The presence of nitrosamines in cigarette smoke condensate (CSC) could not be demonstrated by extraction, distillation, and steam distillation of CSC. Neutral fractions of CSC were reduced with  $\text{LiAlH}_4$  to form hydrazines of any nitrosamines present which were then reacted with 5-nitrosalicylaldehyde and 3,5-dinitrobenzoylchloride to form the corresponding hydrazones and hydrazides, respectively. Thin-layer chromatography did not reveal the anticipated nitrosamine derivatives. In providing controls for this research, 12 nitrosamines were synthesized and characterized.

2. Composition of Tobacco Leaf. In contract research at the Research Triangle Institute, Durham, North Carolina, techniques were developed for separating the hexane extract of flue-cured tobacco into classes of compounds based on polarity and molecular size. The method developed, which depends upon counter-current distribution for polarity separation, and gel permeation chromatography for molecular size separation, is reproducible, nondestructive, and permits quantitative recovery. Its applicability has been demonstrated by the isolation and identification of several compounds and classes of compounds by following it with conventional chromatographic techniques (column, thin-layer, and gas/liquid chromatography). Among the substances isolated were glycerides, solanesol esters, sterol esters (including cholesterol esters), solanochromene (plastochromenol-8), solanesol,  $\alpha$ -tocopherol,  $\beta$ -amyrin, and sterols (including cholesterol). Molecular weight determinations indicated that 70% of the nonbasic hexane extract was below 800 in molecular weight.

Under contract with the University of Kentucky Research Foundation, Lexington, 12 trace elements - La, Br, As, Cr, Ag, Se, Zr, Sc, Sb, Zn, Co, and Fe - were determined in burley tobacco leaves, stalks, ash, 19 different brands of cigarette tobacco, 2 brands each of cigar and pipe tobacco, and the cigarette papers from 12 different brands of cigarettes. Two more elements, Au and Hg, were determined after radio-chemical separation. The data obtained reveal that La, Sb, and Zn are present in lower amounts in tobacco stalks than in tobacco leaves. The reverse is true for Br, Co, and Se. Except for Sb and Fe the concentrations of other elements are about the same in tobacco leaves and cigarette tobacco. Values for Sb and Fe are higher in cigarette tobacco than in tobacco leaves. The higher values for Fe in cigarette tobacco are thought to be due to the contact of the shredded tobacco with the iron and steel machinery used in cigarette manufacturing. La, Co, and Zn are higher, and Sc and Sb lower in pipe and cigar tobacco, but the levels of other elements seem to be about the same as in cigarette tobacco. The amounts of Br, Sc, and Sb are lower in cigarette paper than in tobacco; the amounts of Zn, Cr, Co, and Ag are about the same, while that of Se seems to be slightly higher. While Hg levels in both tobacco leaves and cigarettes are about the same, there is a distinct difference in the gold levels. The gold concentration in cigarette tobacco is about eight times less than in tobacco leaf. However, in both cases the levels are so low (fraction of ppb) that they are probably of no physiological importance. Preliminary data on the elements remaining in tobacco ash indicate that most of the Br is lost upon ignition. About 75% of La, 50% of Se, 90% of Zn, 96% of Co, and 73% of Sb also is lost upon ignition.

## B. Chemical and Physical Investigations to Improve Products

1. Biological Assay. Assays, over a period of 50 weeks by the mouse-back-painting method, are conducted under contract by the Roswell Park Memorial Institute, Orchard Park, N. Y. This method, due to replications and length of test period, requires preparation of large quantities of test material at EU. The material being tested can be grouped into two categories, fractions of smoke condensate and concentrated solvent extracts of tobacco leaf. Results to date have drawn attention to one member of each category as containing the most biological (tumorigenic) activity. These are (a) the highly polar group of neutral substances from cigarette smoke and (b) the least polar fraction, the petroleum ether (hexane) extract of flue-cured tobacco leaf.

2. New Methods of Bioassay. In contract research at the University of Kentucky Research Foundation, Lexington, fundamental data are being obtained on biological systems and on their response to tobacco constituents in evaluating their use as a bioassay procedure to replace or augment the mouse-back-painting method. A radioassay procedure was developed for following the metabolism of labeled benzo(a)pyrene by rat liver homogenates. Several systems are concerned with in vitro tissue culture. Others are concerned with responses in animals. For example, a special stain was developed to permit more rapid and accurate counting of epithelial cells in determining the cell turnover rate of the bronchial epithelium of mice.

Chromosome changes in the marrow cells of young rats are being evaluated following intravenous injection of authentic carcinogens. Experiments on the absorption of ingested benzo(a)pyrene revealed that a linear increase in the dosage resulted in an exponential increase in concentration in the mammary gland and adipose tissue. No trend was noted for liver, kidney, spleen or thymus.

3. Cigarette Modifiers. A comprehensive final report on contract research has been submitted by Houdry Laboratories. During the course of the work, the effect of nine possible cigarette additives on the composition of the vapor phase of cigarette smoke was measured. Vapor phase constituents determined, as a means of evaluating the possible utility of the additives, included a number with ciliostatic or other toxic property (e.g., formaldehyde, acetaldehyde, acrolein, hydrogen cyanide, carbon monoxide, nitrogen oxides). Additives that produced the most noticeable effects on the vapor phase of cigarette smoke included sodium nitrate which induced significant increases in concentration levels of acrolein, acetaldehyde, acetonitrile, nitrogen oxides, hydrogen cyanide and carbon monoxide; nickel oxalate which raised hydrocarbon concentrations; and a ferric oxide-sugar mixture which markedly reduced concentration levels of carbon monoxide and other volatiles. It was also noted that a significant factor influencing levels of smoke gases was the degree to which the smoke was diluted with air. Dilution effects were dependent upon the porosity of the cigarette paper, and the length of the cigarette, among other things.

In cooperative research at the University of Kentucky, Lexington, blended cigarette tobacco was treated with a variety of chemical additives. These were selected on the basis of their anticipated chemical activity, as a result of thermogravimetric analysis of the treated tobacco, or on the basis of a preliminary screening whereby a substantial lowering of total particulate matter (TPM) was obtained. Cigarettes made from these treated tobaccos were smoked and the condensates analyzed for TPM, nicotine, benzo(a)pyrene, phenol, o-cresol and m-,p-cresol. On the basis of marked changes in the levels of certain of these smoke components, tobaccos treated with 10%  $\text{NH}_4\text{VO}_3$ , 10%  $\text{NaHCO}_3$  and 10%  $\text{KClO}_3$  were proposed for bioassay. The smoke from cigarettes from 12 experimental tobaccos which differ in the levels of nicotine, total sugars, polyphenols and sterols also was analyzed. Except for nicotine, no definite relationship was noted between any of these leaf constituents and the composition of the smoke.

Research at EU has shown that the pH of cigarette smoke can be shifted widely by the use of appropriate cigarette additives. The proportions of nicotine and pyridine in the vapor and particulate phases of smoke are altered markedly by shifting smoke pH and selective removal of nicotine at alkaline smoke pH can be demonstrated. The mechanisms of inhibition of dehydrogenase activity by smoke are more complex than previously believed.

4. Pyrolytic Products. The efficacy of the hexane soluble fraction of tobacco in producing, on pyrolysis, benzo(a)pyrene and other aromatic hydrocarbons has been demonstrated. Estimates of benzo(a)pyrene content in



pyrolysates indicate that nearly two-thirds of the amount produced during tobacco pyrolysis may be attributed to the hexane soluble components of leaf, although these components comprise only 6% of leaf weight.

Menthol, a cigarette additive, and caffeic acid, a tobacco leaf constituent, have been shown to produce, on pyrolysis, a number of compounds present in cigarette smoke.

Pyrolysis of amino acids at the University of Kentucky Research Foundation continued to show that the products contain a number of neutral and basic compounds, including polynuclear hydrocarbons.

#### G. Microbiology and Toxicology

Phosphate esters of carbohydrates and nucleotides have shown promise as indicators of metabolic activity differences between fermented and cured tobaccos.

Two new methods for the separation of biologically important phosphate esters and related compounds have been developed. One of these, column chromatography on poly-N-vinyl pyrrolidone, removed the highly colored and fluorescent materials which have, in the past, caused considerable interference difficulty when working with tobacco extracts. Some kinetic and molecular weight studies have been made on a partially purified tobacco peroxidase. Studies on the ultrastructure of the tobacco leaf during processing have been initiated.

#### D. Technology - Process and Product Development

1. Production of Smoke Condensate. Ten kilograms of cigarette smoke condensate have been fractionated for the second phase of the bioassay research under contract at Orchard Park, N. Y. Further subfractionation of weakly acidic and neutral fractions is in progress. Preliminary tests indicated biological activity in a non-polynuclear-aromatic hydrocarbon of the neutral constituents of smoke condensate.

Laboratory facilities for manufacturing and smoking experimental cigarettes to obtain condensates for chemical and biological studies have been established at the University of Kentucky. More than 100,000 cigarettes were manufactured during the previous six-month period. In the Smoke Condensate Preparation Laboratory 63,300 cigarettes of various types were smoked and more than 1,200 grams of condensate were recovered. A short-term study to evaluate the acute toxicity of smoke condensates from two tobacco varieties, Old Virginia Bright Leaf and Coker 319, which were cured using two different systems was initiated. Because of the high nicotine content of these tobaccos both whole and denicotinized condensates were evaluated. As expected, even at the 50 mg/week dosage level, the whole condensates proved to be too toxic. However, preliminary results indicate that the denicotinized condensates can be used at the desired dose level of 150 mg. per week.

2. Production of Extracts of Tobacco Leaf. Large quantities of tobacco were extracted with petroleum ether, chloroform, acetone, ethanol, methanol and water. Concentrated extracts have been analyzed by the mouse-back-painting



method at Orchard Park, N. Y. The results of the tests show that the main tumor-promoting activity is concentrated in the least polar extract, the petroleum ether fraction. This activity decreases rapidly as the polarity of the extracting solvent increases, i.e., the alcohol extracts show little activity. Consequently, efforts were then made to fractionate the petroleum ether extract in order to concentrate the activity in a smaller fraction. The final adopted method consists of column chromatography on silicic acid and elution with solvent systems of increasing polarity: petroleum ether, petroleum ether-benzene mixtures, petroleum ether-ether mixtures, methanol, and acetic acid. This procedure yields a series of 15 fractions, which are presently submitted for bioassay.

#### RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

##### A. Chemical Composition, Physical Properties and Structure

Research to determine the constituent(s) responsible for the objectionable odor in cigar butts has revealed the presence of several hundred compounds. By using polar and nonpolar columns the butt condensate may be fractionated and separated by gas chromatography into individual components for spectroscopic identification. Eighteen compounds have been identified along with several tentative identifications. No single component appears responsible for the butt aroma at this time. Attempts to quantitatively estimate the amount of butt condensate have met with no ultimate success. Analysis of water content of cigars, unsmoked cigar butts, and smoked cigar butts have been fairly consistent. The determinations of organic residuals in smoked cigar butts has had little success, possibly since gravimetric methods were employed. The rate of cooling of cigar butts was determined to see if there was any low temperature pyrolysis occurring which might give rise to compounds having objectionable odors. The volatile amines of cigar butts were trapped as their hydrochlorides. Attempts to analyze them by gas chromatography has produced only inconclusive data. A novel smoke injector was devised which can inject whole or filtered smoke from any puff of a cigar into a gas chromatograph for analysis.

##### B. Chemical and Physical Investigations to Improve Products (no current research)

##### C. Microbiology and Toxicology (no current research)

##### D. Technology - Process and Product Development (no current research)

## Publications -- USDA and Cooperative Programs

## RPA 709 - REDUCTION OF HEALTH HAZARDS INVOLVED IN USE OF NONFOOD FARM PRODUCTS

Chemical Composition, Physical Properties and Structure

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS  
FROM FIELD CROPS

Chemical Composition, Physical Properties and Structure

Peck, R. L., Osman, S. F. and Barson, J. L. Cigar butt aroma. I. A preliminary study of cigar butt headspace vapors. Tob. Sci., 13, (1969), 38-39.



## MAPLE SAP AND SIRUP UTILIZATION

USDA and Cooperative Program

Problem Area		: Scientist Man-Years		
		: F.Y. 1969		
No.	Title and Activity	Intra- mural	Extra- mural	Total
401	New and improved forest products			
	Chemical composition and physical properties	0.3		
	Microbiology and toxicology	1.0		
	Technology - process and product development	1.9		
	TOTAL	3.2		

## Problems and Objectives

Maple syrup producers, in an area encompassing 14 states from Maine to Minnesota and south to Virginia, are largely full-time small farmers. Under proper conditions, maple syrup can be a six-week seasonal crop not in competition with other farm activities and with a per acre value equal to or exceeding that of other farm products. Only 3% of the available trees are being tapped for sap production. Extensive stands of untapped sugar maple trees are located in agriculturally depressed areas and these trees represent a good potential source of cash income in these areas. The production of maple specialty items is another potential source of cash income for the farmer or producer. Sustained effort is required to obtain the information needed so that all operations for the production of high-quality maple syrup and other maple products can be conducted in a predictable, efficient manner.

Major objectives of the research are:

1. Improving technology and reducing cost of sap collection and processing.
2. Improving quality of maple syrup and other maple products.
3. Developing new maple products.

## Progress - USDA and Cooperative Programs

### RPA 401 - NEW AND IMPROVED FOREST PRODUCTS

#### A. Chemical Composition and Physical Properties

The flavor profiles of maple syrups from five different maple-producing areas in the U.S. have been obtained by a recently-developed GLC procedure. The patterns of the profiles were remarkably uniform, each containing more than 90% of the total number of compound-indicating peaks in all the profiles. Every profile contained all of the important, known compounds related to maple flavor.

The original GLC procedure was made more practical. It was modified so that the highly sensitive GLC flame ionization detector could be used. By this change sample size for analysis has been reduced from one gallon to 1/2-pint. This also reduced the solvent cost and time required to make a profile.

#### B. Microbiology and Toxicology

Studies of static storage of maple sap under direct ultraviolet (UV) irradiation showed that at normal storage temperatures (45-55°F.) the penetration of germicidal UV rays into the sap was not adequate to prevent bacterial growth at depths of over 2 feet. It was found that the bacterial population

in stored sap could be reduced and controlled at a low level by recycling sap from the bottom of the tank to the sap surface, thus continually renewing the surface of the sap exposed to the germicidal rays from overhead UV lamps. Bacterial populations were decreased more than 99% by recycling sap stored at 55°F. for 6 hours at a pump rate which recirculated all the sap in the tank every 4 hours. Lower temperatures depressed the lethal effect of the UV rays, but a repetition of the previous study carried out at 35°F. still decreased bacterial population 40% in 4 hours.

Efforts were made to adapt the resazurin dye reduction test (which is used to quickly estimate microbial contamination levels in raw milk) to the needs of central sap evaporation plants. It was found that even though sap can support large microbial populations it is a relatively poor culture medium and consequently the metabolic activity of the contaminating organism is too low to produce the rapid blue to pink color change required for a positive test reaction. The time required for a positive resazurin test reaction has been reduced from 4 to 1 hour by adding nutrient broth to sap and incubating the mixture 1/2 hour before adding the dye solution.

Sanitizing reverse osmosis membranes with acidified hypochlorite maintained the reverse osmosis unit in good sanitary condition during idle periods up to 3 days in length, but difficulties developed with washing residual hypochlorite flavor from the membrane. A new chlorine dioxide sanitizer is being tested. It is as effective as the hypochlorite and is readily rinsed from the membranes.

### C. Technology - Process and Product Development

The Eastern Utilization Reverse Osmosis Concentrator (EUROC) was field-tested in 1968 at a commercial centralized maple syrup plant. Fifty percent of the water was removed from maple sap by the reverse osmosis (RO) unit and the concentrate was then finished to syrup by the plant's regular procedure. This syrup could not be differentiated from the syrup made by the plant's regular procedure. The RO procedure reduced the energy cost of making syrup 54%.

An improved membrane module for RO became available in late 1968. Tests with EUROC showed it to be 50% more permeable than the previous one. For field testing in 1969, one-fourth of the membrane area of EUROC was replaced with the new membrane modules. With the new modules, 60% of the water was removed from the sap. Again no deleterious flavor effect was detected.

Stirred maple sugar is a special form of maple sugar which has high solubility properties due to the way in which it is made. Unfortunately, it "cakes" badly due to its relatively high moisture content. The addition of one of the commercially available forms of micron-sized silica in the amount equal to 1% of the weight of the sugar will keep the product free-flowing.

## Publications - USDA and Cooperative Programs

## RPA 401 - NEW AND IMPROVED FOREST PRODUCTS

Chemical Composition and Physical Properties

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